Informing Landscape-Scale Management of the Greater Horseshoe Bat *Rhinolophus ferrumequinum*

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Declaration

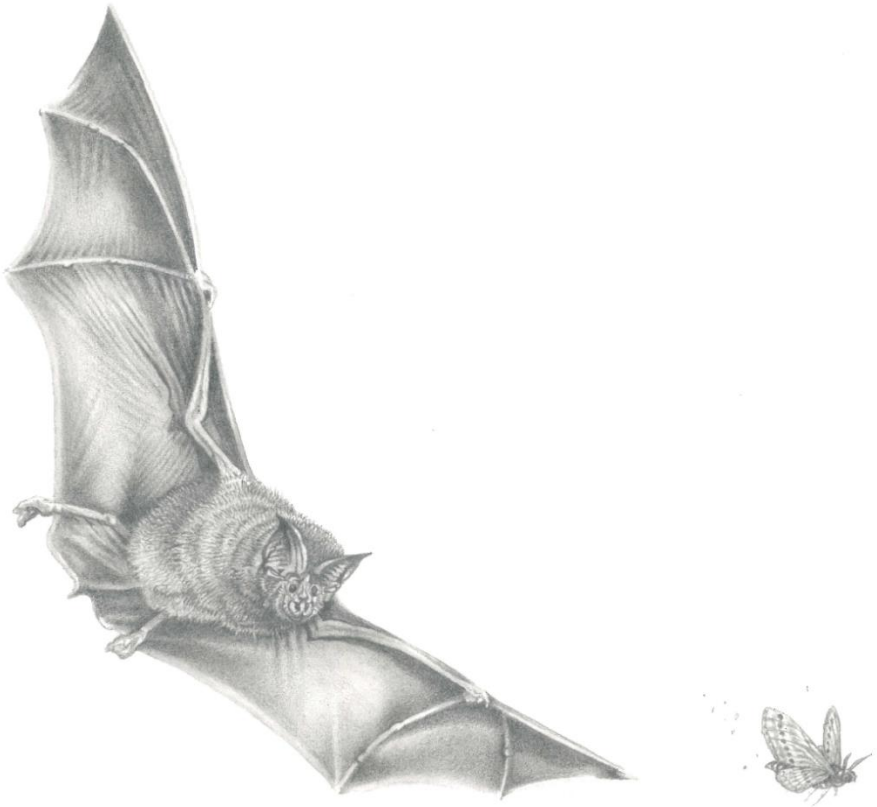
I hereby declare that this thesis has been composed by myself and that it embodies the results of my own research, which have not been and will not be submitted in whole or in part to another University for the award of any other degree. Where appropriate, I have acknowledged the nature and extent of work carried out in collaboration with others.

……

Domhnall Finch

Do mo mháthair is m'athair,

le buíochas ó chroí



Abstract

Global land use is changing at an unprecedented rate and has been identified as a key driver of habitat loss, fragmentation and species decline in the natural environment. Understanding how land use influences spatial patterns in species abundance, and habitat connectivity at a landscape scale is critical for the survival of wildlife populations. The focal species of my research is the rare greater horseshoe bat (*Rhinolophus ferrumequinum*), which was once widespread across southern England and Wales. However, owing to changes in agricultural land management and the expansion of urban areas, its range has contracted considerably over the last century. Using a series of ecological techniques, including a novel predictive modelling approach, field experiments and social network analysis, this thesis aims to identify which ecological factors affect their activity and movement at a landscape scale. The work also provides conservation practitioners the ability to identify the locations of these impacts, pinch-points, in the wider environment; where strategic planning and mitigation measures can be applied to increase their overall occurrence and abundance in the wider environment.

Using a field experiment, I examined how traffic noise can influence the relative activity levels of free-living bats. Overall, I showed that traffic noise can significantly reduce the activity levels of *R. ferrumequinum*, as well as other bat species, along linear feature. Using a separate field experiment, I determined that the sonic spectrum had a greater negative effect on bat activity than the ultrasonic spectrum. These results therefore suggest that the mode of action is likely to be through general deterrence and avoidance rather than through the masking of echolocation calls. *R. ferrumequinum* are widely considered to be dependent on linear landscape features such as woodland edges and hedgerows. My research supported this view, and highlighted the particular importance of treelines, which were associated with greater activity than even sympathetically managed hedgerows. However, an important novel finding from my research was that about a third of all activity recorded at paired detectors was derived from the middle of fields. It is therefore important to consider these more open habitats, as well as

hedgerows, treelines and woodland edges, when designing and conducting ecological impact assessments for future developments.

Bats use the landscape at a large spatial scale, and responses to any particular challenge (such as a new lighting scheme or urban development) are likely to depend on their context within a landscape. For example, the disruption of a commuting route is likely to have a greater impact where only one suitable route exists, compared with scenarios where there are numerous alternatives. To understand better how *R. ferrumequinum* interacts with the British landscape, I therefore created models of predicted functional connectivity around four maternity roosts using Circuitscape software. Using non-invasive static bat detectors as a method of ground validation, I created robust models predicting *R. ferrumequinum* movement; which allowed the identification of pinch-points in the landscape, either those areas limiting species movement or highly important for the species conservation.

While most research, and the work of ecological practitioners, focuses on the maternity season, hibernation ecology has received much less attention. Yet given that horseshoe bats are known to move roost location frequently during the hibernation period, it is important to understand more about this behaviour. Using social network analysis, I demonstrate that adult males are significantly more central (connected to a higher number of individual bats) in the network during the hibernation period. I found that movements between hibernacula were associated with both age and degree centrality of individual bats, with those more geographically isolated hibernacula playing an important role for the movement of certain individuals at a landscape scale. This highlights that despite low activity in some of these smaller roost locations, they are a conservation priority to decrease the risk of fragmentation and loss of connectivity within the wider landscape.

The results of my meta-analysis, which was based on 22 studies, demonstrated the significant negative effects endectocides on Aphodiinae dung beetles. My results suggest that ivermectin

has the highest negative effect on the abundance of both adult and large Aphodiinae dung beetles. However, contrasting results were observed for dung beetle occurrence, with adult beetles showing an attraction to dung with endectocides and larvae showing the complete opposite, with poor survival rates and impaired development. Over time this could have significant negative effects on dung beetle populations.

The results of this thesis indicate that the landscape-scale conservation of *R. ferrumequinum* is complex. Considerations need to be given to a suite of factors ranging from the prey items they consume to the physical habitat structures which they utilise. From this research, specific locations and features which have impacts on their movement and activity can be identified, allowing the outputs to be used by decision-makers as a tool to inform local management strategies. The prioritisation of conservation activity for the species can be aided by spatially-explicit models, such as the one I developed using Circuitscape, which bring together multiple input layers to create outputs readily interpretable to practitioners. However, to achieve a successful outcome for this priority species, collaborative efforts from many stakeholders, across boundaries, are required.

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Chapter 1

General Introduction

Globally land use is changing at an unprecedented rate, with the primary drivers being agricultural changes and urbanisation (Millennium Ecosystem Assessment 2005a; Millennium Ecosystem Assessment 2005b). These changes significantly impact ecosystems at different spatial scales, leading to a more fragmented landscape and reducing its permeability for species (Vanbergen *et al.* 2005; Lawler *et al.* 2006; Fahrig 2007; Hendrickx *et al.* 2007; Firbank *et al.* 2008; Barbaro & van Halder 2009). This landscape fragmentation is one of the leading causes of global biodiversity loss and has resulted in the progressive disappearance of local populations across a range of taxonomic groups; including birds, mammals, invertebrates and plants (Ceballos & Ehrlich 2002; Flynn *et al.* 2009).

Due to the rapidly growing demand for food and natural resources, the rate of change in land-use activities and the associated impacts on biodiversity are set to continue accelerating throughout the globe. These losses in biodiversity are currently having significant effects on ecosystems services which critically contribute to human well-being (Haines-Young & Potschin 2010; Howe *et al.* 2014). With human populations continuing to grow, there is increasing demand for these services. Against this background, the challenges of reducing and reversing the impacts of landscape fragmentation can be achieved, but will require major alterations in political and institutional practices (Millennium Ecosystem Assessment 2005a).

Understanding how these changes and pressures influence spatial patterns in species abundance and composition on a landscape scale is critical for the survival of wildlife populations (Gorresen, Willig & Strauss 2005; Fahrig *et al.* 2011). Here I review landscape scale ecology and conservation, focusing on the status and ecology of the greater horseshoe bat (*Rhinolophus ferrumequinum*).

1.1 Landscape ecology and conservation

1.1.1. Key terms and concepts

The term “landscape ecology” was coined by the Carl Troll (1939); it principally uses spatial analysis from geographers in combination with functional approaches from ecologists (Forman & Godron 1986). It is used to describe connections between spatial patterns and ecological processes at broad scales (Turner 1989), with a landscape described as an area that is spatially heterogeneous (see se1.1.2ction 1.1.2) in at least one factor of interest (Forman 1995). A simple description of landscape ecology which incorporates both its broad scope and interdisciplinary relationships can be broken down into three parts (Forman 1983):

1) The spatial relationship among landscape elements, or ecosystems;
2) The flow of energy, mineral nutrients, and species among the elements; and
3) The ecological dynamics of the landscape mosaic through time.

These three points illustrate that the interactions between a species and its landscape are affected by the composition and structure of its environment (Watt 1947), and that the strength of these interactions vary with distance between individual organisms and locations (Turner & Gardner 2015). All of these interactions are dependent on scale (Wiens 1989). Current research has illustrated that local scale (the same scale or smaller than the abundance/factor of interest) results are not necessarily scalar and may not be related to broader scale environmental issues such as climate change or habitat fragmentation (Fahrig 2005; Turner & Gardner 2015). Landscape ecology studies have shown that maintaining a diverse range of species and viable populations can be achieved through a well-connected heterogeneous landscape that contains high quality habitat characteristics with different spatial configurations and compositions (Wiens 1995; Hendrickx *et al.* 2007; Haslem & Bennett 2008).

1.1.2. Landscape heterogeneity

Landscape heterogeneity can be described as the combination of landscape composition and the spatial positioning (configuration) of habitat features (Fahrig & Nuttle 2005; Oliver *et al.* 2010; Fahrig *et al.* 2011). These elements play a crucial role within the functioning of the ecosystems and affect biodiversity, ecosystem services and social interactions both within and between species (Fahrig 2007; Fraterrigo, Pearson & Turner 2009; Fahrig *et al.* 2011; He, Maldonado-Chaparro & Farine 2019).

1.1.2.1. Landscape configuration and composition

The term landscape configuration refers to the non-random spatial arrangement of habitat characteristics and structure in the landscape (Forman 1995). Many landscape metrics, such as patch size, edge density, patch shape, linear connectivity/barriers and interspersion of patches, are used to measure the impacts of landscape configuration on species composition and movement. In species-specific studies, landscape configuration is mainly used to examine to the effect of habitat fragmentation and connectivity within the landscape (Bennett et al., 2006).

Landscape composition refers to the number of different elements and their relative proportions present within a landscape mosaic (Bennett, Radford & Haslem 2006), whilst it is not making any reference to the spatial attributes/arrangement of these different elements within the landscape (McGarigal, Cushman & Ene 2012). The composition of elements within each landscape can be classified in a number of ways. For example, as land cover type (woodland, arable, cattle-grazed fields), from a vegetative perspective (coniferous versus deciduous trees cover) or structurally (height of vegetation etc.; Fuller, Trevelyan & Hudson 1997). The composition of these classifications within a landscape affects habitat availability and hence the distribution or occurrence of target species. For example, the dispersal of species through a landscape may be enhanced by the provision of connective elements, or increased predator behaviour may be observed in response to an increase in the availability of edge

habitats. The significance of these effects particularly impact fragmented landscape that consists of small isolated habitat patches (Kupfer, Malanson & Franklin 2006; Kennedy *et al.* 2010).

1.1.2.2. Landscape mosaic

A landscape ecology approach is not only concerned with the heterogeneity of the landscape but also with the interactions with the surrounding environment and spatial extent at which it is measured (Thies, Steffan-Dewenter & Tscharntke 2003; Dunford & Freemark 2005; Turner & Gardner 2015). Two patches that are indistinguishable in terms of size, shape, quality and relative connectivity to other patches will respond differently to ecological processes depending on the surrounding habitat mosaic. The occurrence of a species within a patch can often be explained by the characteristics of the elements both within the core patch and the surrounding landscape, the influences of these elements varies geographically and among species (Steffan-Dewenter *et al.* 2002; Prugh *et al.* 2008; Lintott *et al.* 2016). This is particularly true for species groups, including many mammals (Thornton, Branch & Sunquist 2011), that require a wide range of heterogeneous habitats within the landscape (Dunning, Danielson & Pulliam 1992). Oliver *et al.* (2010) illustrate that landscape heterogeneity can support stability in butterfly population by providing a wide range of resources that can help buffer population fluctuations. In addition, the different elements within a landscape mosaic required by a species may be used during different temporal scales and may only be needed for one particular part of its life cycle (e.g. Pope et al., 2000). Overall, this demonstrates that what happens at a specific locations is often influenced by the surrounding environment. This knowledge is essential when designing conservation management plans as the surrounding landscape and the connectivity within it can significantly influence the outcome of the results.

1.1.2.3. Connectivity

Connectivity within landscape ecology refers to the degree to which a landscape facilitates or impedes the movement of an individual between habitat patches and different resource types

(Dunning, Danielson & Pulliam 1992; Forman 1995; Tischendorf & Fahrig 2000). In ecological terms, there are two different types of connectivity within the landscape: structural and functional. Structural connectivity describes the degree to which habitat patches are physical linked, whereas functional connectivity relates to the actual movement of an individual between habitat patches (Turner & Gardner 2015). The cost incurred by individuals in travelling between two specific patches is known as the resistance value (Urban & Keitt 2001; Baguette *et al.* 2013).

Connectivity can influence habitat patches in three main ways (Murphy & Lovett-Doust 2004):

1) Impeding or improving the rate of species dispersal/colonisation;
2) Facilitating the introduction of new invasive species; and
3) Allowing access to alternative habitats.

This concept of connectivity is fundamental for meta-populations (source-sink) to be able to survive within changing landscapes, and can be illustrated by delayed re-colonisation of populations to areas that become locally extinct (e.g. Verboom et al., 1991). This is particularly true for bat species as they often require links to highly dispersed resources within a landscape and are often strongly associated with specific habitat features such as mature treelines/woodland edge. The loss of connectivity can negatively affect bats through the creation of a 'barrier effect' which can imped their movement between habitat patches (Stone, Jones & Harris 2009; Berthinussen & Altringham 2012b; Mathews *et al.* 2015; Azam *et al.* 2016; Fensome & Mathews 2016). This can have significant implications for how species utilise the landscape (forage, commute, reproduce), with some resources becoming unavailable due to a loss in connectivity (e.g. Henry et al., 2007). From a conservation and management perspective, adding or protecting key areas of connectivity is likely to have significant positive effects on the landscape/species, particularly when the number of elements within the composition of that suitable habitat is low (Andren 1994; Fahrig 1997). A fundamental understanding of these issues

can allow for better management of the wider landscape and the conservation of rare, highly mobile species that require a variety of connected habitat types to survive.

1.1.3. Landscape conservation and management

One of the overarching aim of conducting landscape scale ecological research is to put into practice the correct management plans to sustainably conserve biodiversity (Wiens & Moss 2005). To do so, key concepts must be understood and acted upon such as accounting for meta-population dynamics, heterogeneity and connectivity within the landscape. To create effective management plans targeted objectives, conservation measures and monitoring approaches must be defined from the most up-to-date scientific knowledge of ecological process and species traits from the outset. Unfortunately many management plans fails to achieve their full potential due to poor definition of the objectives and applications of on the ground conservation strategies (Fazey *et al.* 2006).

Effective conservation of habitats and species not only considers the impact of a single patch but also takes into account the mosaic of landscape around it (Turner & Gardner 2015). This is essential as a single patch management plan which implements the most modern and well-designed conservation measures may fail to deliver its targets due to the ecological degradation of its hinterlands (Lindenmayer *et al.* 2008). For bats, particularly rare and sensitive species such as the *R. ferrumequinum*, protecting only a roost site is insufficient as foraging grounds, commuting routes and areas of social interaction are also essential for viable meta-populations (e.g. Ransome, 1996).

Both anthropogenic and natural pressures can directly impact the carrying capacity of habitats through degradation and/or fragmentation. This can impact the sustainability of a meta-populations within the landscape leading to smaller more vulnerable populations going extinct, with a consequent loss of genetic diversity. One way to combat such extreme events is to create or restore suitable habitats (foraging/breeding grounds, commuting routes etc.) for the species

within the wider landscape (Hodgson *et al.* 2011), reducing population isolation and maintaining meta-populations (Hanski 1998; Hanski 1999). Two of the most effective and successful landscape scale conservation management plans that have been developed and implemented within the UK are the 'special projects' within the English Countryside Stewardship Scheme for the stone curlew (*Burhinus oedicnemus*; Bealey *et al.* 1999; Green, Tyler & Bowden 2000) and the cirl bunting (*Emberiza cirlus*; Stanbury *et al.* 2010; Davies *et al.* 2011). These projects illustrate that successful practical conservation can be achieved when the wider landscape is taken into account and species specific knowledge and targets are implanted in the correct manner. In addition, these types of landscape scale conservation and management plans can have significant positive effects on non-target species within the landscape as well (MacDonald *et al.* 2012).

1.1.4. Predictive tools used in landscape scale conservation

Loss of habitat connectivity is a critical global issue (Worboys, Francis & Lockwood 2010). The sizes and quality of habitat patches and their relative fragmentation can alter connectivity over both spatial and temporal scales (Ewers & Didham 2006). This variation makes effective conservation actions difficult to develop and implement, particularly when incorporating the movement of species across corridors within the landscape into management plans (Kool, Moilanen & Treml 2013).

To aid the understanding of environmental interactions on species occurrence and distribution, spatial computer algorithms and software packages have been developed over the last decade (Scheller *et al.* 2010). These use habitat variables and species occurrence/distribution data to create predictive Habitat Suitability Models (HSM) and interactive maps. This allows us to effectively map the habitat niche species occupy and deliver valuable information about species habitat and social network requirements at a landscape scale (Guisan & Zimmermann 2000; Hirzel & Le Lay 2008), especially for rarer and more mobile species such as bats (Bellamy, Scott

& Altringham 2013; Razgour *et al.* 2013; Bellamy & Altringham 2015; Razgour *et al.* 2016). Both Gorresen, Willig and Strauss (2005) and Bellamy, Scott and Altringham (2013) have illustrated that different environmental variables can influence bat species distribution depending on the scale and resolution at which they are examined.

Within the literature HSM have been labelled using various terminology including Species/Habitat Distribution Models, Resource Selection Functions or Ecological Niche Models (Boyce & McDonald 1999; Guisan & Zimmermann 2000; Rushton, Ormerod & Kerby 2004; Peterson *et al.* 2006). Some of the latest methods for HSM and connectivity modelling include the use of Geographical Information Systems (GIS; Erickson, McDonald & Skinner 1998; Danks & Klein 2002; Gontier 2007) and include such packages as MaxEnt (Phillips, Dudík & Schapire 2004), Conefor (Saura & Torne 2009), Corridor Design (Majka, Jenness & Beier 2007), Zonation (Moilanen, Kujala & Leathwick 2009) and Circuitscape (McRae & Shah 2009). Some HSMs have the ability to be transferred between geographic regions using the same species criteria within the model but different environmental data (Peterson, Papes & Eaton 2007). The resulting maps are easily interpretable communication tools. Their uses include highlighting to decision-makers where to focus and create conservation strategies to generate connectivity and mitigate negative impacts of human developments (Abbitt, Scott & Wilcove 2000; Johnson & Gillingham 2005).

1.1.4.1. Graph theory models

The graph theory (Harary 1969) has been used in various scientific disciplines for solving issues relating to network routing, social network analysis and the flow of energy between points. More recently it has been used in conservation biology to examine habitat connectivity and corridors (Urban & Keitt 2001; Beier, Majka & Spencer 2008; McRae *et al.* 2008; Urban *et al.* 2009). The output of the models produced represent continuous surface graphs and networks that are comprised of both nodes and edges. Nodes are connection points which, in ecological

terms, could represent habitat patches, individual bats or roost locations. Edges represent connections between two nodes within the model network (e.g. movement of animals from one place to another or their association between each other; Urban & Keitt 2001). Each edge is weighted based on the strength of its connection or interaction between nodes (McRae *et al.* 2008). When used for connectivity models, these are described as cost surface models where the highest value is equal to a high cost of movement rather than a high suitability within any given landscape (O'Brien *et al.* 2006). These types of computer models can bring useful insights into functional connectivity within landscape ecology rather than the focus just being on structural connectivity (Lookingbill *et al.* 2010).

One of the most widely used methods for connectivity modelling involving the graph theory is the Least-Cost Path (LCP). The LCP is based on the movement of a species between two nodes (habitat patches) for which the species accumulates a cost as it moves away from its source node (Adriaensen *et al.* 2003). The costs incurred depend on the specific habitat features/characteristics encountered and the dispersal capabilities of the species being examined (Avon & Bergès 2015). Using this methodology, a single path is identified which provides least resistance and the highest probability of survival when travelling the route (Russell, Swihart & Feng 2003; Beier, Majka & Spencer 2008; Poor *et al.* 2012). One of the major criticism of this type of connectivity modelling is that it implies that the species knows exactly where this one path is and that all individuals travel the same path of least resistance (Theobald 2006; Avon & Bergès 2015). In reality this may not allow good predictive movement of individuals between nodes, and it does not take into account the existence of other paths which allow for random dispersal depending on an individual's behaviour (Belisle 2005; McRae & Beier 2007). To counter this, recent advances in calculating resistance distances between nodes has been delivered through using the circuit theory (Guillemin 1953) and the random walk theory (Doyle & Snell 1984) in conjunction with the graph theory. This has been implemented using Circuitscape software (McRae & Shah 2009).

Circuitscape describes landscape connectivity through the application of the circuit theory to graphs created using the graph theory. This application keeps the original graph structure with linked nodes but it substitutes the graph edges with electrical resistors (Urban & Keitt 2001; McRae *et al.* 2008). This function allows a theoretical landscape to be represented as a conductive surface and permits an electrical current to pass through it from the source node outwards to all other nodes within the circuit, much like the inverse of the cost surface in the graph theory (McRae *et al.* 2008; Urban *et al.* 2009). The current can therefore travel along multiple potential dispersal pathways at once, taking into account both random movements of individuals and redundant pathways, with each pathway equally probable (McRae 2006; McRae & Beier 2007; McRae *et al.* 2008).

The current denotes dispersal of species, with nodes of low resistance being represented by environmental variables that are most permeable within the landscape (McRae *et al.* 2008). Current densities can be calculated at each node and linked to the relative movement of a species within the wider landscape. When Circuitscape is calculating current density it not only takes into account the environmental variables present within that specific node but also the influence of the variables in the neighbouring nodes (McRae *et al.* 2008). This feature can help highlight critical corridors or "pinch points" and identify potential barriers (McRae *et al.* 2008; McRae & Shah 2009; Braaker *et al.* 2014; Pelletier *et al.* 2014). It has been argued that the outputs of Circuitscape identify dispersal corridors more accurately and reliably compared to other landscape connectivity models (Poor *et al.* 2012; Roever, van Aarde & Leggett 2013b; McClure, Hansen & Inman 2016).

1.2 Greater horseshoe bat

1.2.1. Species description and distribution

R. ferrumequinum (Schreber 1774) is one of 77 bat species that are included in the Rhinolophidae family (Harris & Yalden 2008). These species are distributed throughout Europe, Asia and Africa (Csorba, Ujhelyi & Thomas 2003), with higher diversity found in more tropical regions. Five species of Rhinolophidae are known to occur in Europe, two of which are recorded within the British Isles, *R. ferrumequinum* and *Rhinolophus hipposideros* (Dietz & Kiefer 2016). One of the main distinguishing features of this family is its 'nose-leaf', the lack of the tragus within its ears and its highly mobile ears, all features that are related to their echolocation system. These species produce high constant frequency calls, which are emitted through their nostrils, and detect changes in Doppler shift of the returning echoes to orient themselves in their environment and locate their prey. For *R. ferrumequinum,* the frequency of its calls is between 79 and 84 kHz, depending on their distribution (Jones & Ransome 1993; Russ 2012).

R. ferrumequinum are a medium sized bat in relation to the other Rhinolophidae and the largest in Europe (Dietz & Kiefer 2016). It has an approximate head and body lenght of 56 – 68 mm and a wingspan of 330 – 395 mm. The species has an approximate forearm length of 50.6 – 59.0 mm and a weights of between 13 – 34 g, with males on average being slightly smaller than females (Harris & Yalden 2008). *R. ferrumequinum* has thick fur all over its body, which for adults is a brown to grey-brown colour with dorsal fur that darkens and turns reddish brown with age. The fur of the juvenile *R. ferrumequinum* is grey in colour, and changes to buff brown in the first year (Dietz & Kiefer 2016).

In Europe, *R. ferrumequinum* distribution stretches from Greece and Portugal in the south and to Slovakia across to Britain in the north. The latter is the most north westerly extent of its distribution (Figure 1.2.1). Globally they are listed as Least Concern on the IUCN red list database (Aulagnier *et al.* 2008), but in Europe they are Near Threatened (European Environment Agency

2020). The species is protected under at least 13 different national and European legislations, including the Wildlife and Countryside Act (1981) (as amended), Bonn (1979) and Bern (1982) Convention, as well as the Habitats Directive (Council Directive 92/43/EEC on the Conservation of natural habitats and of wild fauna and flora' (1992)). Its more northerly European populations have dramatically declined over the last 100 years (Hutson & Mickleburgh 2001). In Britain, *R. ferrumequinum* are considered to be extremely rare and has a core population restricted to South West England and Wales (Figure 1.2.2). However, in recent decades there has been a recent expansion of both population size and species range. There are small colonies now recorded in North Wales and along the southern coast of England as far east as West Sussex

Figure 1.2.1: Map of the current greater horseshoe bat (*Rhinolophus ferrumequinum*) distribution throughout Europe and northern Africa.

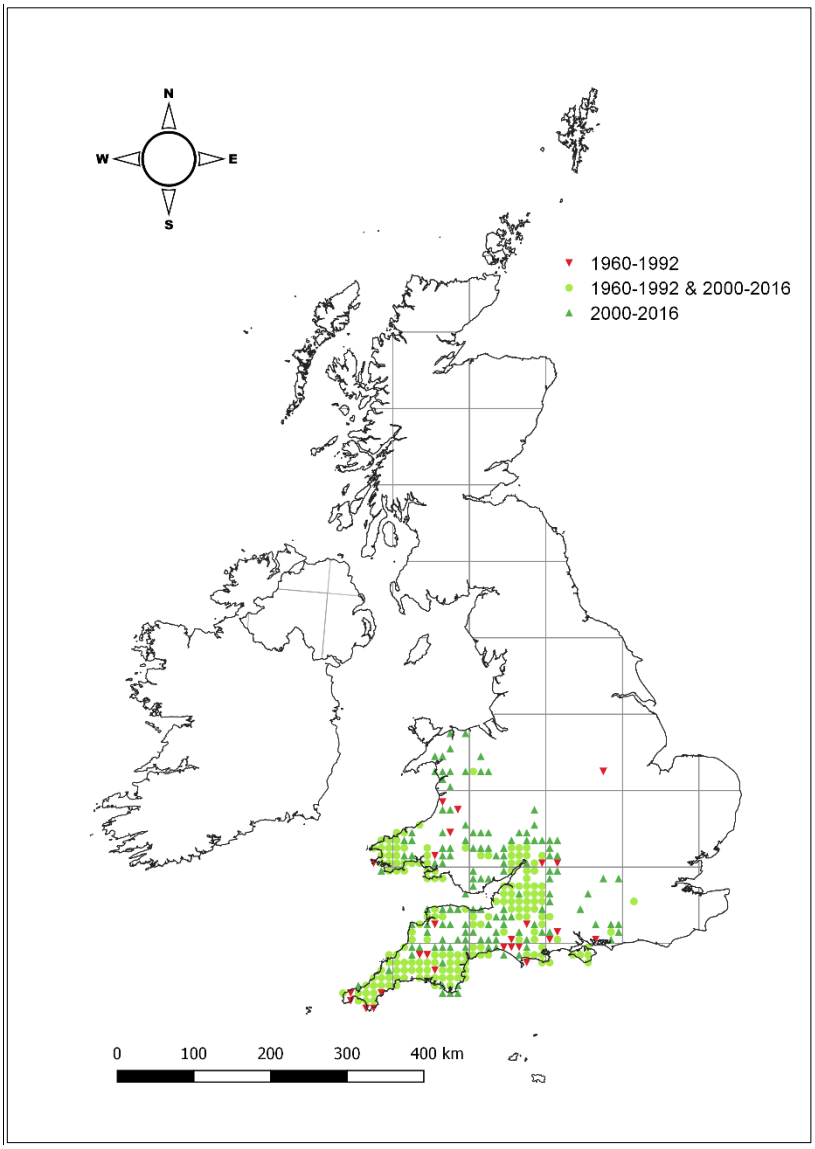


Figure 1.2.2: Current and past distribution of greater horseshoe bat (*Rhinolophus ferrumequinum*) in Britain (Mathews *et al.* 2018).

1.2.2. Population Status of the Greater Horseshoe Bat in Britain

Historical studies have shown that *R. ferrumequinum* had a much wider distribution in the past, with records occurring as far east as London and Kent up until around 1900 (Stebbings 1989; Harris 1995). An estimate of their population at the start of the 1900s suggest that they numbered around 300,000 individual (Stebbings & Arnold 1989), although this figure is disputed (Ransome 1989). Population estimates, based on low sampling effort, from the latter half of the 20th Century suggest that there were approximately 2,200 *R. ferrumequinum* bats in Britain (Stebbings & Griffith 1986). This estimate increased to approximately 4,000 in 1993 (Hutson

1993). It has to be noted that some additional *R. ferrumequinum* roosts were found between these periods which were not included in prior estimate. At the start of the 21st century the population was estimated to be 6,600 (Battersby 2005). However, the most current population estimate is 12,900 (Mathews *et al.* 2018).

Due to the long term protection and monitoring (National Bat Monitoring Programme) of this species in Britain accurate population trends have been obtained (Bat Conservation Trust 2016). The trend analysis takes into account data from 32 maternity roosts and 231 hibernacula in both England and Wales between 1999 and 2015. The results indicate that overall numbers within maternity roost are up 126% from 1999, which is an annual increase of 5.2% (Bat Conservation Trust 2016). This positive trend is also reflected in hibernation counts with an overall 113% increase in total numbers comparted to 1999; this equates to an annual increase of 4.8% for hibernating bats. However, recent (2012-2015) trend analysis reveals that there has been a slight decrease in numbers (Bat Conservation Trust 2016). When this trend is examined further, the decline seems focused on the Welsh rather than the English population.

1.2.3. Foraging, habitat use and prey items

Given the legal protection afforded to *R. ferrumequinum*, there is a considerable body of work which has investigated habitat use and their diet. In a broad context, *R. ferrumequinum* mainly foraging around pastures (predominately cattle grazed pastures), deciduous woodlands, riparian habitats and hedgerows/treelines (Ransome & McOwat 1994; Jones, Duvergé & Ransome 1995; Flanders & Jones 2009; Dietz, Pir & Hillen 2013; Dietz & Kiefer 2016; Ancillotto *et al.* 2017; Froidevaux *et al.* 2019). Most radio-telemetry studies in the South West of England show that the main core foraging and commuting sustenance zone around a *R. ferrumequinum* maternity roosts is approximately 4km for adults and 2km for juveniles (Billington 2002; Billington 2003). However, *R. ferrumequinum* do travel and forage regularly at much larger distances from the roost (9-12km) (Ransome & Hutson 2000; Billington 2008).

Flanders and Jones (2009) showed that in the South West of Britain *R. ferrumequinum* spent relatively equal amounts of time utilising pasture and deciduous woodland habitats in spring (42.1% and 37.5%, respectively). However, the emphasis of these habitats switched during the summer months with more activity occurring over pasture compared to deciduous woodland (Duvergé & Jones 1994). This shift in dependence of different habitat features throughout the season has been linked to the availability and abundance of key prey items within them. The importance of well-developed hedgerows and mature treelines to *R. ferrumequinum* has been highlighted by a number of studies, not only as critical networks of commuting routes and potential roost locations but also as shelter belts and key food resources (Ransome & McOwat 1994; Ransome 2000; Flanders & Jones 2009; Dietz, Pir & Hillen 2013; Dietz & Kiefer 2016; Froidevaux *et al.* 2019).

Most of the evidence on the diet of *R. ferrumequinum* in Europe is based on dietary analysis of British colonies. *R. ferrumequinum* is a selective feeder that specifically targets large prey items, predominately Arthropoda (Jones & Rayner 1989; Jones 1990; Ransome & Hutson 2000). Both Lepidoptera and Coleopteran species are the main prey items of *R. ferrumequinum* when available, with Tipulidae, Trichoptera and Ichneumonid parasitic wasps also being preyed upon (Jones 1990). By volume Lepidoptera contributes 41% of the total diet of *R. ferrumequinum* throughout the year and Coleoptera 33%. Of these Coleoptera, 29% were beetles that are associated with cow pats, primarily *Aphodius spp.* (Jones 1990).

There is considerable seasonal variation in dietary composition. Both Jones (1990) and Ransome (1996) show the main prey items per month:

- April and May – mainly Geotrupes and Melolontha with some Tipulids and Ichneumonid;
- June and July – dominated by Lepidoptera (between 60 and 85%) with *Aphodius spp*. more frequent in late July;
- August and September – mainly *Aphodius spp*.; and

- October to March - Geotrupes, Tipulids and Ichneumonid.

Overall, the results of these studies illustrate the specialised selection of prey items by *R. ferrumequinum* and the ability for it to adapt its foraging behaviour between seasons and habitat type, which is aided by its sophisticated echolocation technique.

1.2.4. Roosting ecology

R. ferrumequinum uses a variety of different roost structures, depending on the time of year and reproductive status. The main types are maternity and hibernation roosts, although mating, transitional and night roosts are also used (Park, Jones & Ransome 2000; Ransome & Hutson 2000; Flanders & Jones 2009).

During the summer (May - September), maternity roosts are located in a variety of different structures, including the attics within houses, old barns, churches and heated cellars. In the more southerly areas of its European distribution it also uses caves (Ransome & Hutson 2000; Debernardi & Patriarca 2007; Flanders & Jones 2009; Pavlinic & Dakovic 2010; Maltagliati, Agnelli & Cannicci 2013; Dietz & Kiefer 2016). In the northern half of their distribution across Europe, maternity roosts are almost exclusively found in buildings with the average colony size of between 20 and 200 individuals (Ransome & Hutson 2000; Hutson & Mickleburgh 2001; Dietz & Kiefer 2016). These roosts are usually in in dark roof spaces that reach high temperatures during the day (some in excess of 40^{o}C).

Within the winter hibernation period (October – May), *R. ferrumequinum* are found in cooler underground sites, usually caves and mines with temperatures usually between 6 and 12^{o}C (Ransome 1971; Hutson & Mickleburgh 2001; Dietz & Kiefer 2016). However, the requirements of temperature and humidity within hibernacula, depends on the age, sex and the overall condition of the bat (Ransome 1968; Ransome 1971; Ransome & Hutson 2000). Arousal from torpor – usually at dusk – occurs at intervals ranging from 1 to 18 days depending on the time of year and the ambient temperature within the hibernaculum (Ransome 1968; Ransome 1971;

Park, Jones & Ransome 1999; Park, Jones & Ransome 2000; Ransome & Hutson 2000). During arousal bats usually complete some type of grooming and stay within the cave system, suggesting that although foraging does occur during the hibernation period it is unlikely to be the principal purpose of arousal (Ransome 1971; Park 1998; Park, Jones & Ransome 2000; Ransome & Hutson 2000).

Temperature is not the only factor in determining where *R. ferrumequinum* hibernate. Three different types of hibernacula are described in the literature that are dependent on the sex and age of individual bats (Ransome 1968; Ransome 1971; Ransome 2000; Ransome & Hutson 2000):

> **Type 1:** Usually containing first year and older immature *R. ferrumequinum* of both sexes.
>
> **Type 2:** Where mainly 2nd and 3rd year bats are found (although it may also but contain 1st year bats and adult males). The adult males usually form clusters and on occasions solitary adult females are observed in Type 2 hibernacula.
>
> **Type 3:** Used as a breeding territory for the same adult male over a number of years. These are usually small isolated exposed hibernacula which are mainly occupied in autumn and again in spring where adult females join the male for a period. Increasing evidence suggests that mating occurs at these sites. If the Type 3 hibernacula is a large cave the male may reside there for the entire winter period.

Ransome and Hutson (2000) notes that Type 3 hibernacula are the most abundant and widespread within a population, followed by Type 2 and then Type 1 hibernacula. It is important to note that multiple alternate locations of each type (1-3) occur within the same geographical area of a landscape and are utilised by the same population of *R. ferrumequinum* during hibernation.

Despite these descriptions and observations, very little has been statistically tested regarding the social associations and movement of hibernating *R. ferrumequinum*. Questions remain regarding whether, unlike during the maternity season, males play a more active role in *R. ferrumequinum* society, or if adult females form tight knit bonds and how long do these bonds last for. Additionally, very little information has been gathered on how often or when individuals move between hibernation sites and how this might impact the interactions between individuals at a population level. Examining these movements and social interactions can allow for a better understand of the species ecology and would be critical for the conservation of the species at a landscape scale.

1.2.5. Human induced threats

1.2.5.1. Roost disturbance

Caves used by *R. ferrumequinum* can come under pressure from disturbance due to rock extraction, recreational caving, and use as tourist attractions (Johnson, Brack & Rolley 1998; Ransome & Hutson 2000; Hutson & Mickleburgh 2001; Gaisler & Chytil 2002). Due to safety concerns numerous underground sites formerly used by *R. ferrumequinum* have been permanently sealed (Tuttle & Taylor 1998; Johnson, Wood & Edwards 2006), though over recent decades there has been increasing use of grilles to allow bats continued access. Disturbance by conservationists during the hibernation period, for example through the ringing of bats or the checking of ring numbers, may also pose a threat to bats (Hutson & Mickleburgh 2001; Gaisler & Chytil 2002). Each disturbance event during a hibernation period may cause arousal of the bat from torpor using up vital energy supplies. Thus, if disturbance causes arousal too frequently it could have implications on their survival through the hibernation period (Ransome & Hutson 2000; Hutson & Mickleburgh 2001; Gaisler & Chytil 2002).

There are also pressures on the type of buildings used as for maternity roosts by this species through demolition, renovation or development. (Hutson & Mickleburgh 2001; Dietz & Kiefer 2016). Direct destruction or alteration of roost buildings are not the only factors to reduce

roosting opportunity for this species. Remedial timber treatments, historically, organochlorine timber treatment caused significant bat mortality (Mitchell-jones *et al.* 1989). The illumination of roost exits can delay emergence reducing foraging time. In extreme circumstances, artificial lighting can also act as deterrent which may cause the permanent loss of the roost site (Downs *et al.* 2003; Boldogh, Dobrosi & Samu 2007). This is a particular concerns for roosts within public buildings such as churches but also in caves which are exploited for tourism (Ransome & Hutson 2000). Both natural and non-native predators pose significant risk to *R. ferrumequinum* colonies. For example, domestic cats can cause both disturbance to activity patterns and emergence times, as well as *R. ferrumequinum* behaviour, e.g. greater clustering within roosts (Ancillotto, Venturi & Russo 2019).

1.2.5.2. Habitat loss and degradation

At the northern edge of its range, habitat alteration is thought to be one of the major causes of decline in *R. ferrumequinum* (Hutson & Mickleburgh 2001). Given the importance of woodlands, permanent pastures and linear features for *R. ferrumequinum* and their prey (Duvergé 1996; Billington 2008; Froidevaux *et al.* 2019), the loss, fragmentation and degradation of these habitats is of particular concern (Ransome & McOwat 1994; Flanders & Jones 2009; Boughey *et al.* 2011; Cizek *et al.* 2012; Dietz, Pir & Hillen 2013; Burgio *et al.* 2015; Caro *et al.* 2016). However, efforts have been made in the past to enhance these features specifically for *R. ferrumequinum* through Countryside Stewardship Schemes (Duvergé & Jones 2003).

Additionally, urbanisation, infrastructure, traffic noise and street lighting may act as a barrier to the movement of bats through the landscape (Downs *et al.* 2003; Stone, Jones & Harris 2012; Day *et al.* 2015; Hale *et al.* 2015; Luo, Siemers & Koselj 2015; Mathews *et al.* 2015). Roads also present a direct collision risk to bats, with *R. ferrumequinum* mortalities being recorded several counties in Europe (Fensome & Mathews 2016).

1.2.5.3. Prey availability

On a broad scale, pesticide use is documented as a serious threat to insectivorous predators (De Reede 1982; Agosta 2002; Henderson *et al.* 2009; Kunz *et al.* 2011; Hallmann *et al.* 2014). There are particular impacts on the larvae of favoured prey items for *R. ferrumequinum* (tipulids, noctuid moths, melolonthid beetles), these are considered pests due to the large quantities of the grass roots they consume within agricultural fields (Ransome & Hutson 2000; Jackson & Klein 2006; Peck, Olmstead & Morales 2008; Toth *et al.* 2010). As with many predators the build-up of toxins due to the consumption of prey items containing sub-lethal doses can have detrimental impacts on their populations through the reduction in their body condition and effects on their reproduction. This has been extensively recorded in birds of prey and in particular Barn Owls (*Tyto alba*) (Mendenhall, Klaas & McLane 1983; Newton, Wyllie & Asher 1991; Newton, Wyllie & Dale 1997).

The impact of chemical use is not just restricted to direct control of pest species. There are also non-target effects, for instances from the use of endectocides (to combat intestinal worms and ecto-parasites) in cattle and sheep (Ransome & Hutson 2000; Hutson & Mickleburgh 2001; Dietz & Kiefer 2016). Endectocides are broad spectrum drugs administered by injection, use of a bolus, or applied as a drench poured onto livestock. Residues of the products are found in the dung of treated animals. Research has showed that this can have detrimental impacts on dung fauna, especially at the larvae stage (Wall & Strong 1987; Hempel *et al.* 2006; O'Hea *et al.* 2010; Sutton, Bennett & Bateman 2014).

1.3 Study aims and thesis outline

Despite a growing awareness of the negative impacts of increased rates of urbanisation and changes to agricultural land management on biodiversity, most studies in this field focus on single issues. In this thesis, I try to disentangle the various causes of habitat degradation for a rare bat species. Bringing together issues that affect the availability of prey items, how social

structure of overwintering bats may have implications for its conservation, and I analyse the anthropogenic barrier effects in the species landscape.

In particular, the aims of this thesis are to:

- Investigating the impact of traffic noise, both the sonic and ultrasonic spectrums, on relative bat activity and feeding records. Using a playback experiment this research aims to identify which specific aspect of traffic noise impacts bat activity but how this is caused, e.g. avoidance behaviour, echolocation masking. The study discusses the potential need for traffic noise to be considered in all future Environmental Impact Assessments and road schemes (Chapter 2).
- Assess how different land management regimes can influence species diversity and abundance along linear features and in the middle of field boundaries. These results will potentially give insight to facilitate the formulation of management strategies to identify and protect suitable foraging and commuting features within the landscape for the benefit of a variety of bat species (Chapter 3).
- Using predictive software, examine how different landscape features can influence the movement of *R. ferrumequinum* from their roost locations to potential foraging areas. Detecting which features can act as barriers to their movement and which increase permeability into the wider landscape; while identifying where practical mitigation measures would be best placed to increase functional connectivity into the wider landscape and have the highest conservation impact (Chapter 4).
- Assess the long-term social structure of hibernating *R. ferrumequinum* populations by monitoring species attributes using social network analysis. Identifying which type of individuals are likely to travel between hibernation locations between study years and which individual bats have the highest social connectivity within wintering populations of *R. ferrumequinum*. These results will have important implications on the conservation

of wintering *R. ferrumequinum* populations and highlight the significance of alternate types of hibernation locations (Chapter 5).

- Investigate contradicting literature sources to identify the effect of multiple endectocides on both life stages, adult and larvae, of aphodiine dung beetles. These findings will have important implications for our understanding on how different endectocides affect the occurrence and abundance of aphodiine dung beetles in the wider agricultural landscape and how this can influence the availability of prey items for *R. ferrumequinum* (Chapter 6).

Chapter 2

Traffic noise playback reduces the activity and feeding behaviour of free-living bats.

An adapted version of this chapter has been published as:

Finch, D., Schofield, H., and Mathews, F., 2020. Traffic noise playback reduces the activity and feeding behaviour of free-living bats. Environmental Pollution, p.114405.

2.1. Abstract

Increasing levels of road noise are creating new anthropogenic soundscapes that may affect wildlife globally. Bats, which form about a third of all mammal species, are sensitive bioindicators, and may be particularly vulnerable because of their dependency on echolocation. However, few studies have solely focused on this potential impact, with many suggesting its influence when examining roads as a whole but cannot differentiate its effects, and as such what can be done to mitigate it. Here we present the first controlled field experiment with free-living bats. Using a Before-After-Control-Impact phantom road experimental design, we examine the impacts of traffic noise on their activity and feeding behaviour. Disentangling the impacts of traffic noise from other co-varying exposures such as habitat quality, the experiment demonstrates a significant negative effect on the activity of each of the five, ecologically different, species (genus for *Myotis* spp.) examined. This suggests that the results are widely applicable. The negative effects are largely attributed to noise in the sonic spectrum, which elicited aversive responses in all bat species tested, whereas responses to ultrasound were restricted to a single species. Our findings demonstrate that traffic noise can affect bat activity at least 20m away from the noise source. For *Pipistrellus pipistrellus* and *Pipistrellus pygmaeus*, feeding behaviour, as well as overall activity, was negatively affected. We demonstrates the type of impact traffic noise can have on bat assemblages, signifying that Ecological Impact Assessments are needed wherever there are significant increases in traffic flow, and not just when new roads are built. Further research is required to identify effective mitigation strategies, to delineate the zone of influence of traffic noise, and to assess whether there is any habituation over time.

2.2. Introduction

The exponential growth of the human population and the rapid increase in global urbanisation has profound implications for wildlife. Networks of roads have been built through the natural environment, posing barriers to the movement of animals through collision risk and habitat loss, as well as causing indirect effects on habitat quality that can compromise foraging and commuting (Forman 2003; Keller & Largiader 2003; Fensome & Mathews 2016). While road densities have only increased by approximately 10% between 1990 and 2011 in OECD countries (Organisation for Economic Co-operation and Development), traffic densities have increased by 55% (OECD Publishing 2013). This has led to a global growth in anthropogenic noise pollution and the creation of new soundscapes, which can alter how animals use their primary sensory systems to detect or recognise cues to forage, communicate, find mates and avoid predators (Brumm & Slabbekoorn 2005; Swaddle *et al.* 2015; Senzaki *et al.* 2016). Species diversity, occupancy, reproductive success and survival (e.g. Goodwin & Shriver 2010; Halfwerk *et al.* 2011; Wiącek *et al.* 2015), can be affected through the masking of both territorial and predatory alarm calls (Mockford & Marshall 2009; Templeton, Zollinger & Brumm 2016; Nelson *et al.* 2017), and by eliciting avoidance behaviour and stress responses (McClure *et al.* 2013; Troïanowski *et al.* 2017; Hastie *et al.* 2018).

Compared to birds, little research has been undertaken with bats to disentangle the impact of traffic noise from overall effects, which could include a combination of light, sound and air pollution as well as habitat changes (e.g. Berthinussen & Altringham 2012b; Pourshoushtari *et al.* 2018; Claireau *et al.* 2019b; Medinas *et al.* 2019). However, recent experimental evidence, based on captive animals, using acoustic playback systems in an enclosed environment suggests that traffic noise can reduce the feeding success of *Myotis myotis*, *Myotis daubentonii* and *Antrozous pallidus* (Schaub, Ostwald & Siemers 2008; Siemers & Schaub 2011; Bunkley & Barber 2015; Luo, Siemers & Koselj 2015). Both Schaub, Ostwald and Siemers (2008) and Siemers and Schaub (2011) found that traffic noise may mask echolocation calls and the use of passive

listening as a foraging strategy for *M. myotis*, owing to the frequency overlap between the movement of their prey items and the traffic noise being played. In contrast, Luo, Siemers and Koselj (2015) found no evidence of acoustic masking or reduced feeding attention in *M. daubentonii*. Rather, three out of four of the captive bats in their experiment showed signs of avoiding noise below the frequency at which they echolocate, with this avoidance being responsible for reduced feeding success. It was not possible to distinguish whether the reduced feeding success reported by Bunkley and Barber (2015) was owing to call masking or avoidance behaviour.

Given this experimental evidence from captive animals, we have conducted the first controlled field experiments (i.e. outside the laboratory) to test the impacts of traffic noise on free-living bat assemblages. This is vital, as unlike in laboratory studies free-living individuals have a choice of whether they want to be present in the vacinity of the noise source or not. Our study aims to disentangle the effect of traffic noise from other correlated risk factors seen in operational roads, e.g. habitat fragmentation or lighting, by replaying traffic noise in a roadless environment. We are therefore able to examine the local effects of traffic noise in isolation, on a diverse range of bat species. To understand the general consequences of traffic noise for bats, we recorded activity and feeding behaviour for four species with contrasting flight patterns, echolocation and foraging techniques (Russ 2012; Dietz & Kiefer 2016), and also for the genus *Myotis*.

We hypothesised that, at a local scale, the full acoustic spectrum (sonic and ultrasonic spectra combined) of traffic noise would reduce activity for all species recorded. Additionally, we hypothesised that the sonic spectrum (<20 kHz frequency noises) would have a larger negative effect than the ultrasonic spectrum (>20 kHz frequency noises), when played separately, due to bats showing avoidance behaviour rather than their calls being masked. We predicted that the bat assemblage would not become habituated to traffic noise over the period of the experiment, because of the short duration of the exposure, but that feeding activity would also decrease.

2.3. Methods

2.3.1. Traffic noise surveys

Traffic noise from a dual carriageway (A38, Devon, England, 50. 5702555°, -3.6485612°), with a surface cover of asphalt, was recorded at a distance of 3m from the centre of the carriageway closest to the road side verge. Both sonic and ultrasonic frequencies were recorded as wav-files on separate Song Meter SM2BAT+ monitors (Wildlife Acoustics Inc., Maynard, Massachusetts), fitted with SMX-II (sonic) and SMX-U1 (ultrasonic) microphones (for details of the spectral response see Wildlife Acoustics (2019)). Microphones were placed horizontally, side by side, at a height of 1.5m off the ground, and were perpendicular to the centre of the road (i.e. faced the centre). Traffic noise from the vehicles were recorded on a dry, windless day, without a wind guard on the microphones (i.e. under dry asphalt conditions) (Schaub, Ostwald & Siemers 2008), over a period of approximately three hours. Dry conditions were chosen as they have less of an impact when compared to wet conditions (Marimuthu *et al.* 2002). No low or high pass filters were applied during the recordings. The mean vehicle speed, as assessed over 50m, was 110km/h.

To calculate the average recorded pass duration of a vehicle, 50 vehicles were studied. We used Adobe Audition CC (Adobe® Systems, Mountain View, CA, USA) to measure the time at which the vehicles were initially detected by the microphones (assessed by change in frequency compared to background noise) to when the frequency returned to background noise levels. The mean duration of the length of sound from the passing vehicles was 1.4s (SD 0.57). We therefore selected a pass of duration of 1.4s— equivalent to a single passing vehicle — to use in our experiment. The recording deployed was chosen at random from those of this length available. To make the experiment represent real field conditions as closely as possible, we counted the volume of traffic on a dual carriageway for an hour starting at dusk in May. We recorded a mean vehicle pass rate of 26 vehicles per minute. Therefore, this repetition rate was used in the experiment, with the 1.4-second-long passes being dispersed equally across the

minute. To mimic a natural road, the normal recorded background noise of the road was added to the gaps between the vehicle passes, thus avoiding large sections of the recording being artificially blank. As this is the first time free-living bats have been experimentally exposed to traffic noise, we chose to examine whether there was an effect of a single 1.4-second-long pass and background noise for both the sonic and ultrasonic recordings, rather than testing multiple sounds. This approach allows for easy replication in future research. However, other study designs, for example using multiple recordings during playback experiments, could also be considered (e.g. Arroyo-Solís *et al.* 2013). The sonic amplitude of passing vehicles was measured at the roadside over a two-hour period (Precision Gold, NO5CC Sound Level Meter, 30 – 130 dB(A), fast leq), and was found to have a peak of 86dB SPL 3m away from the centre of the carriageway closest to the road side, however it did oscillate as the vehicle arrived at, and continued past, the recording point. These oscillations in noise, caused by the vehicles passing a static point, were included in the playback for the field experiment.

Both our sonic and ultrasonic recordings were created and arranged in Adobe Audition CC, and were played simultaneously through Audacity® (version 2.1.3) (Carnegie Mellon University, PA, USA). Our sonic sound files had a high pass filter set at 1kHz (to avoid damaging the speakers (Schaub, Ostwald & Siemers 2008)) and a low pass filter set at 20 kHz. Our ultrasonic recording had a high pass filter set at 20 kHz and had a sampling rate of 192 kHz (contained frequencies up to 96k Hz; Appendix 2.7.1) (Adobe Audition; digital FFT filter, 2048 points, Blackman window).

2.3.2. Field experiment set up

Before-After-Control-Impact (BACI) designs were used for both the first field experiment in 2017, which examined the impact of the full spectrum of traffic noise (including both sonic and ultrasonic spectra) on bat activity, and for the second field experiment in 2018, which examined the sonic and ultrasonic spectra independently.

The first experiment took place at seven sites around four greater horseshoe bat (*Rhinolophus ferrumequinum*) roost sustenance zones (RSZ; area of land within 3km of a roost) between May and August 2017 in Devon, England. During this first period of data collection both, the sonic and ultrasonic spectra of traffic noises were combined and played back to recreate the full acoustic spectrum of traffic noise. Experimental locations were along linear features (hedgerows/treelines), which were specifically chosen to include different surrounding habitats: grasslands, arable fields, woodland edges and riparian corridors, to enable the results to be generalised as widely as possible. These features had previously had no known disturbance from traffic noise.

Within each of the seven sites, we selected an Experimental location and a Control location. Control locations were at least 500m from any Experimental location to avoid disturbance from noise playback. Each of the Control locations were paired with their respective Experimental locations by choosing locations that had similar habitat features/types surrounding them and were in the same RSZ. For example, if the Experimental location was along a treeline with grass fields on their side, then a Control location along a treeline and by grass fields was chosen.

The protocol at our Experimental locations consisted of two control nights where no traffic noise files were played, followed by three treatment (sound playback) nights. On treatment nights, traffic noise files were played from 30min before sunset for 3.5h. Recordings of bats were made during this time-period on both control and sound treatment nights using four Song Meter SM2BAT+ monitors (Wildlife Acoustics Inc., Maynard, Massachusetts). We placed a single bat detector 20m in front of the first speaker and another 20m behind the last speaker, and the final two were placed in the middle between the three speakers (Figure 2.3.1). In addition, one detector was placed at the Control locations and were set to record in the same way and on the same nights as those at Experimental locations. Both traffic noise recordings were played back on a loop from a laptop computer, through an external D/A-converter (MAYA44 eX 4-in / 4-out

PCIe audio interface, sampling rate 192kHz, ESI Audio, Germany), broadband amplifiers (WPA-600 Pro, Conrad Electronics, Hirschau, Germany) and through three loud-speakers (Avisoft, Speaker Vifa, frequency range 1 – 120 kHz, +/- 9dB) which were set on tripods 1.5m off the ground. Tripods were placed at Experimental locations for both the control and treatment nights. All speakers faced in the same direction (horizontal) and were placed 1.5m out from the linear feature and at 20m intervals along it. Both the ultrasonic and sonic noises were played at the same amplitude on the amplifier to create a peak sonic amplitude of 86dB. The experiment only proceeded on nights when wind speeds were ≤ 11km/h, temperatures were above 10^{o}C at dusk, and in dry conditions. The impact of noise has been shown not to affect the attention available for foraging or the search effort of bats to capture prey in captive settings but a frequency shift has been found in the echolocation call of the greater horseshoe bat (Hage & Metzner 2013; Luo, Siemers & Koselj 2015). It is therefore reasonable to infer that a decline in acoustic activity recorded using bat detectors reflects a true decline in the presence of bats, rather than a reduction in the detectability of the animals due to alteration in their echolocation patterns.

The second experimental period took place between June and August 2018 at six new locations in Sussex and Dorset, England. Because of the generality of the effects observed in the first experiment, the sites were not specifically chosen to be in RSZs (only one was in this category), but all were in locations known to be used regularly by bats. The methods were identical to the first experiment, except that sonic and ultrasonic components of road noise were replayed on separate nights at the Experimental locations: the pattern of playback was one control night, two nights of the sonic components of traffic noise, and two nights of the ultrasonic components of traffic noise. The order in which the noises were played alternated between sites, such that three sites played ultrasonic noise first followed by sonic noise, and three sites did the reverse. This controlled for the effects of treatment order on the findings. All sites were along treelines.

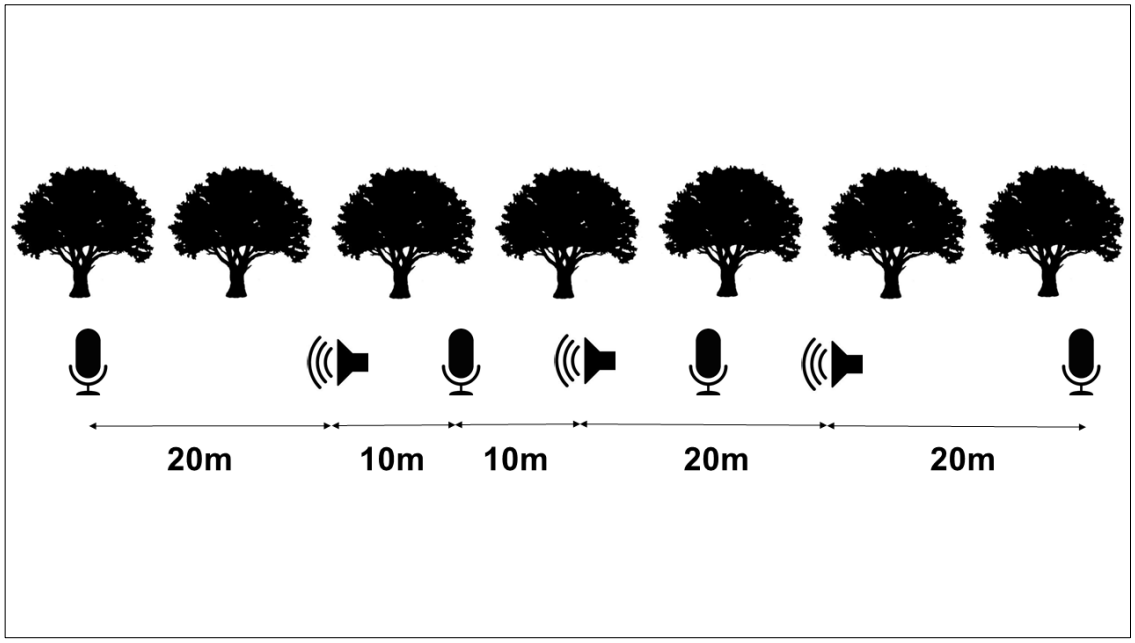


Figure 2.3.1: Depiction of the experimental set up at Experimental locations, with three speakers placed along a linear feature and bat detectors placed between, in front of and behind them to record bat activity.

2.3.3. Bat sound analysis

The analyses of bat activity were conducted using Kaleidoscope software (version 3.1.1; Bats of the UK classifier version 3.0.0; Wildlife Acoustics, Maynard, Massachusetts); all files were also checked manually. Relative bat activity was assessed as the number of bat passes per night during the 3.5 hour survey window (e.g. Jung *et al.* 2012; Charbonnier *et al.* 2014). Individual bat passes were defined as two or more echolocation calls within one second of each other (Fenton 1970; Walsh & Harris 1996a). Some species of *Myotis* bats can be difficult to distinguish, owing to their similar call structures (Schnitzler & Kalko 2001), therefore all *Myotis* species were grouped together for analysis at genus level. The five UK species of *Myotis* that were likely to be recorded during this experiment were *Myotis nattereri*, *Myotis bechsteinii*, *Myotis daubentonii*, *Myotis brandtii* and *Myotis mystacinus*. Feeding activity was defined and recorded as the number of times a feeding 'buzz', the calls emitted while homing in on prey (Kalko & Schnitzler 1989), occurred per night during the survey window. Detailed characteristics of the evolutionary traits of all species examined in this study, including the call structure and their foraging

strategies, can be found in Russ (2012) and Dietz and Kiefer (2016). An example of the call structure of all species groups examined in this study is given in Appendix 2.7.2 – Appendix 2.7.6.

2.3.4. Statistical analysis

Generalised Linear Mixed Models (GLMM), 'lme4' (Bates *et al.* 2015), with a negative binomial distribution, were used to examine the potential impact of traffic noise on total bat activity (11 species); and then separately for the four species and one genus that represented most calls (97%): *Rhinolophus ferrumequinum*, *Pipistrellus pipistrellus, Pipistrellus pygmaeus, Nyctalus noctula* and *Myotis* species. All analyses were completed in R (version 3.3.0) (R Core Team 2016).

We used a BACI approach with the data collected from the first experimental period to assess the impact of playing traffic noise (including both the sonic and ultrasonic spectra) on the number of bat passes per night per detector. This allowed us to ask whether there were differences between Experimental and Control locations that were contingent on time (and hence treatment). The outcome variables were the counts of bat passes per night, and the exposure variables were the temporal variable (control *v.s.* sound treatment nights; fixed factor), the spatial variable (Experimental location *v.s.* Control location; fixed factor) and the interaction between them. The models also included detector position, nested within unique site identities and RSZ identities, as random effects to account for the pairing of the Experimental and Control locations, and the possible non-independence of some of the seven experimental sites that fell within the same RSZ. Using detector position nested within site as a random factor also allowed the models to account for multiple non-independence detectors at each location.

Having established that there were no significant differences at Control locations across time, we then examined whether the amount of recorded activity at the Experimental locations only varied according to the position of the detector relative to the speakers. For this model, the fixed effects were specified as time (control *v.s*. sound treatment nights), detector location (behind

the speakers; in the middle of the speakers; or in front of the speakers; Figure 2.3.1) and their interaction. Detector position, site identity, and RSZ identity, were again specified as random effects. For each species, where significant interactions were identified and suitable data were available, three additional models were created using only data from a single detector location (before the speakers; in the middle of the speakers; or in front of the speakers) at a time. This allowed for time (control *v.s*. sound treatment nights; fixed effect) to be compared at each individual detector location individually to determine if traffic noise impacted bat activity at that specific location. Both site and RSZ were used as random effects in these models. We assessed potential short-term habituation by testing for an interaction between time (control *v.s.* sound treatment nights) and night of treatment nested within time (i.e. from night one to night five). Detector position, site identity, and RSZ identity, were again specified as a random effect.

To examine whether feeding activity was affected by traffic noise playback, binomial GLMMs were created using the number of feeding 'buzzes' recorded per night compared with the number of all other calls (excluding social calls) as the outcome variable. The overall model, examining total feeding activity, used the same fixed and random effects as the initial negative binomial models. If any interactions were observed, we then examined if there was an effect at just Experimental locations, using time (control *v.s*. sound treatment nights) as a fixed factor and the same random factors as the original model for both *P. pipistrellus* and *P. pygmaeus*. Models using feeding buzz data could only be created for *P. pipistrellus* and *P. pygmaeus* owing to the lack of data for other taxa. *Myotis* spp. and *N. noctula* were not examined because there was little feeding activity at baseline, as would be expected in these habitats; and feeding calls are difficult to distinguish for *R. ferrumequinum*.

Finally, we used the data from the second experimental period to assess whether the impacts of road noise resulted from exposure to sonic or ultrasonic components of the sound spectrum. Using a negative binomial GLMM we initially assessed whether there were differences in bat

activity between Control and Experimental locations that were contingent of time (using a temporal variable (control *v.s.* sonic noise *v.s.* ultrasonic noise treatment nights; fixed factor); a spatial variable (Experimental location *v.s*. Control location; fixed factor) and the interaction between them. Then, isolating data from the Experimental locations, we investigated whether nights playing sonic or ultrasonic traffic noise (fixed effect) had an impact on bat activity relative to control nights. The models also included detector position, nested within unique site identities as random effects. Using a binomial GLMM feeding activity were once again examined for the interaction between both the time and spatial variables for total feeding activity, before examining *P. pipistrellus* and *P. pygmaeus* individually at Experimental Locations. Detector position, nested within unique site identities were added to the models as a random factor. If a detector failed to record any bat activity, owing to technically difficulties, the detector position from that site was removed from the analysis. However, similar results were obtained from the BACI analysis when the data were excluded. Given that the data from the Experimental sites were collected under similar environmental conditions, with no significant difference, the data were included in the final analysis. All model residuals were examined to ensure they met the assumptions of the models. Effects were judged as statistically significant when p was less than 0.05.

To test whether environmental conditions, temperature and humidity, significantly influenced nightly bat activity, we included them in our initial analysis when examining treatment night and survey locations (Control and Experimental). For both experiments, we used the same random effects for these negative binomial models, as described above. There was no significant relationship activity and either temperature or humidity and so these variables were not considered further.

2.4. Results

The playback of traffic noise experiment elicited substantial decreases in overall bat activity (Figure 2.4.1). Using the BACI approach, we determined that the total amount of bat activity

recorded at Control relative to Experimental locations differed between noise and control nights (interaction term: p = 0.008). Significantly fewer bat passes were recorded at Experimental locations when traffic noise was being played, whereas at Control locations activity did not differ between control and noise treatment nights (Figure 2.4.2). This provides strong evidence that the reduction in activity was caused by the traffic noise playback. Further summary statistics can be found in Appendix 2.7.7 and Appendix 2.7.8.

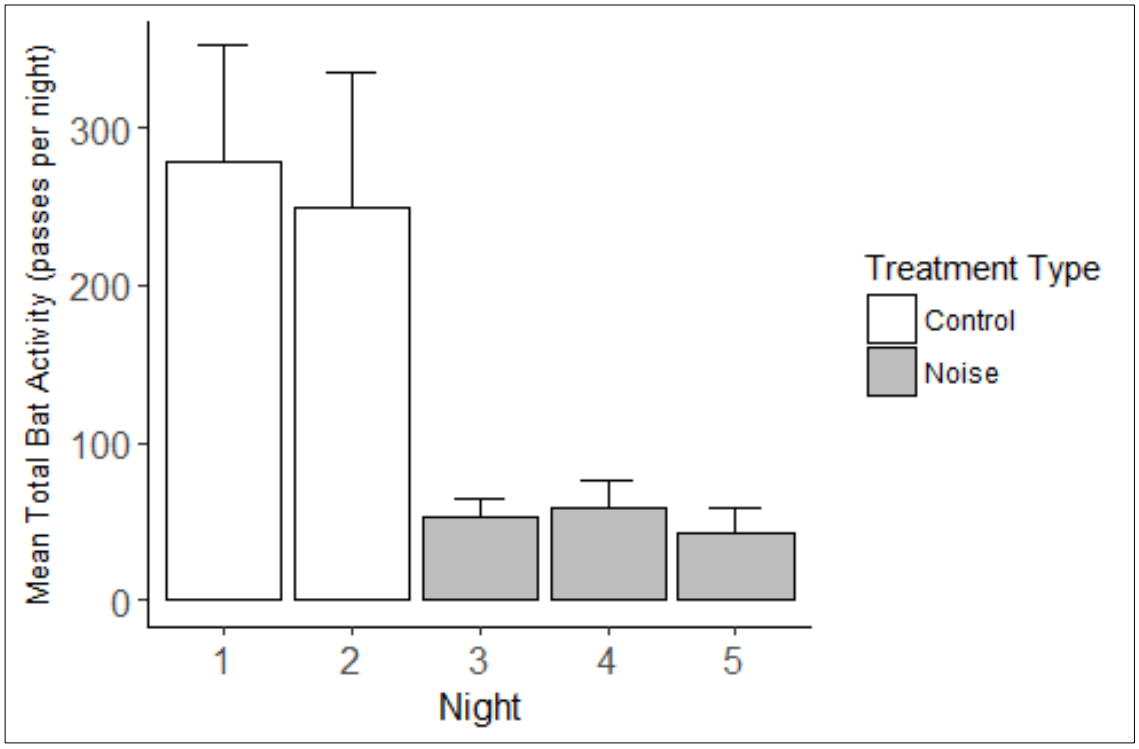


Figure 2.4.1: Mean nightly bat activity (+SE) during control (white) and noise (grey) treatment nights across the seven study sites; graph based on raw activity data. Total number of passes at Experimental locations = 13817 (on control nights: total = 10836, mean = 264, SE = 56; on noise treatment nights: total = 2981, mean = 51, SE = 9).

There was a significant interaction between the amount of bat activity recorded at Control relative to Experimental locations on sound treatment and control nights for *R. ferrumequinum* (interaction term: p = 0.013), *P. pipistrellus* (interaction term: p = 0.032), *P. pygmaeus* (interaction term: p = 0.035) and *Myotis* species (interaction term: p = 0.046) but not for *N. noctula* (interaction term: p = 0.576). When bat activity was examined at Experimental locations only, the number of bat passes for all species/genus was significantly lower on sound treatment compared with control nights: *R. ferrumequinum* (Odds Ratio (OR): 0.23, CI: 0.12 – 0.43, p <

0.001), *P. pipistrellus* (OR: 0.16, CI: 0.09 – 0.29, $p < 0.001$), *P. pygmaeus* (OR: 0.08, CI: 0.04 – 0.17, $p < 0.001$), *N. noctula* (OR: 0.41, CI: 0.24 – 0.71, $p = 0.001$) and *Myotis* species (OR: 0.14, CI: 0.07 – 0.28, $p < 0.001$).

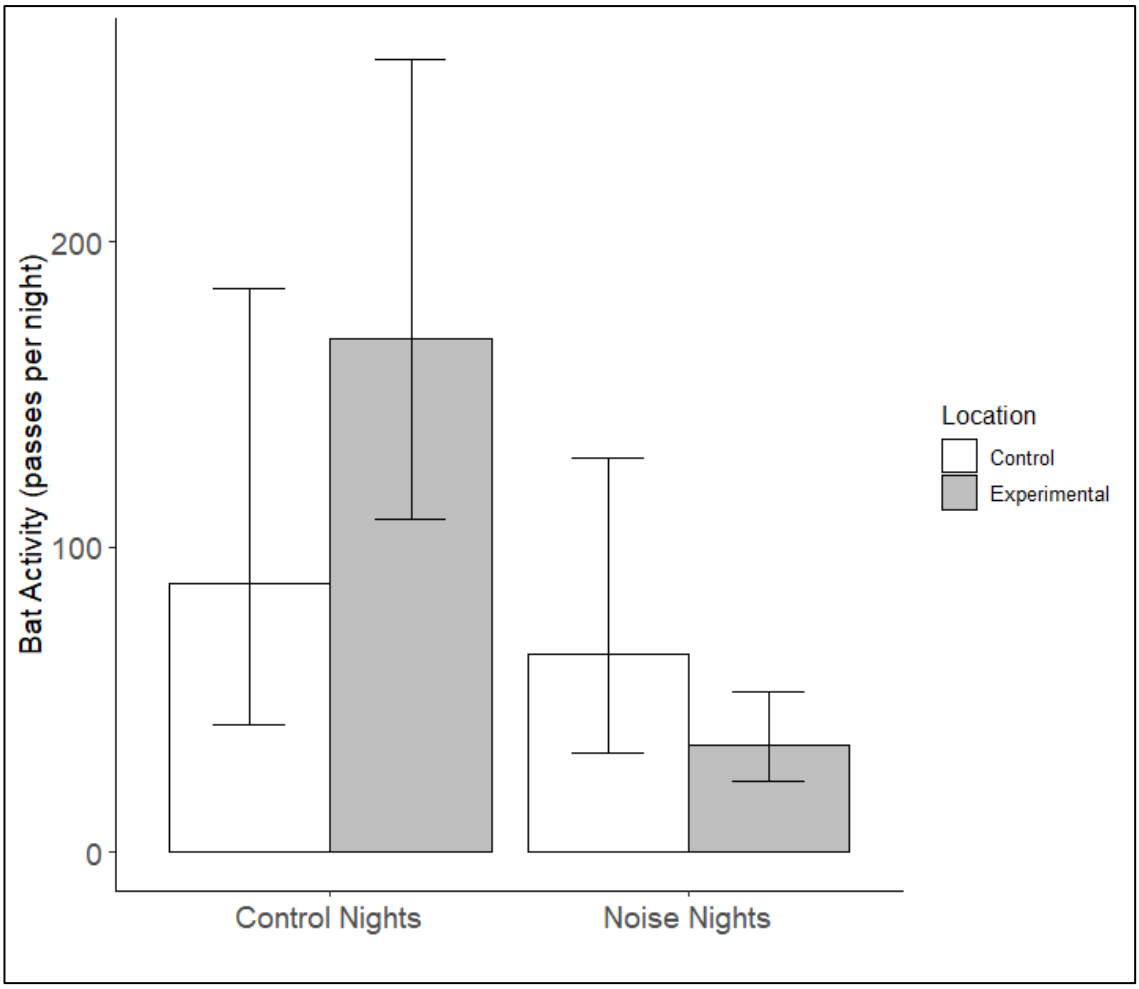


Figure 2.4.2: Predicted bat activity on control and noise treatment nights, at Control (white) and Experimental (grey) locations. Bars show 95% confidence intervals.

Within Experimental locations, significant interactions between detector location and time (treatment *vs*. control nights) were found for, *P. pipistrellus* (interaction term: $p = 0.050$), *P. pygmaeus* (interaction term: $p = 0.037$) and *Myotis* species (interaction term: $p = 0.016$). For *R. ferrumequinum* the interaction term was marginal ($p = 0.072$) and for *N.* noctula the interaction term had a $p = 0.336$). Further models examining individual detector locations could only be created for *P. pipistrellus* and *P. pygmaeus* owing to the lack of data for other taxa. For these species, traffic noise had a significant negative impact on activity at detectors placed both at the

speakers and 20m in front of the speakers (*P. pipistrellus* OR: 0.08, CI: 0.04 – 0.17, $p < 0.001$; OR: 0.18, CI: 0.10 – 0.36, $p < 0.001$, respectively; *P. pygmaeus* OR: 0.03, CI: 0.01 – 0.13, $p < 0.001$; OR: 0.16, CI: 0.01 – 0.47, $p < 0.001$, respectively). There was also a significant reduction in activity 20m behind the speakers for both *P. pipistrellus* (OR: 0.30, CI: 0.10 – 0.94, $p = 0.038$) and *P. pygmaeus* (OR: 0.33, CI: 0.12 – 0.94, $p = 0.038$). We also assessed whether there was evidence of habituation or increased responsiveness at each Experimental location during the noise treatment nights. There was no significant interaction between location and the night on which the noise was played ($p = 0.146$) with activity staying relatively constant over time at Experimental locations.

We examined whether differences between Experimental and Control locations depended on whether it was a noise treatment or control night, and found a significant negative interaction for total feeding activity ($p = 0.025$). At Experimental locations, feeding activity was lower on noise treatment nights compared with control nights for both *P. pipistrellus* (OR: 0.57, CI: 0.47 – 0.69, $p < 0.001$) and for *P. pygmaeus* (OR: 0.53, CI: 0.35 – 0.79, $p = 0.002$).

Having established that road noise playback strongly influenced bat activity, we then tested whether the effects were generated by the sound in the ultrasonic or sonic spectrum. As in the previous experiment, there were no significant differences between bat activity recorded at Control locations. Additionally, at Experimental locations, there were significant differences between control and sound treatment nights for total bat activity (interaction term: $p = 0.043$) and *P. pipistrellus* (interaction term: $p = 0.008$), but not *P. pygmaeus* (interaction term: $p = 0.552$) or *Myotis* species (interaction term: $p = 0.368$). Owing to very low pass rates for *N. noctula* and the rare *R. ferrumequinum*, these species were not analysed further. Both sonic and ultrasonic noise playback had significant negative effects on total bat activity at Experimental locations, though the effect sizes were larger for the sonic treatment (sonic: OR: 0.32, CI: 0.22 – 0.47, $p = 0.001$; ultrasonic: OR: 0.50, CI: 0.35 – 0.72, $p < 0.001$) and *P. pipistrellus* (sonic: OR: 0.34, CI: 0.23

– 0.51, $p < 0.001$; ultrasonic: OR: 0.53, CI: 0.36 – 0.78, $p = 0.001$). For *P. pygmaeus* and *Myotis* species, sonic noise playback reduced bat activity (OR: 0.40, CI: 0.24 – 0.64, $p < 0.001$; OR: 0.34, CI: 0.21 – 0.55, $p < 0.001$, respectively) but ultrasonic noise had no effect ($p > 0.05$)

We identified that there were significant negative interactions between Experimental and Control locations and treatment night for total feeding activity ($p < 0.01$), during the second experiment. Feeding activity appeared to be reduced at Experimental locations on nights with sonic and ultrasonic playback compared with control nights for both *P. pipistrellus* (sonic: OR: 0.65, CI: 0.53 – 0.80, $p < 0.001$; ultrasonic: OR: 0.79, CI: 0.69 – 0.92, $p = 0.001$) and for *P. pygmaeus* (sonic: OR: 0.61, CI: 0.46 – 0.82, $p = 0.001$; ultrasonic: OR: 0.49, CI: 0.39 – 0.62, $p < 0.001$).

2.5. Discussion

We have established, for the first time, that playback traffic noise alone can reduce the activity of free-living bat assemblages, even in the absence of other features associated with roads such as lighting and habitat loss. Reduced activity in response to playback of traffic noise (sonic and ultrasonic spectra combined) was observed for each species that we examined. This includes those species that have markedly different flight heights, speeds, foraging strategies, and with contrasting echolocation patterns. For example species such as *R. ferrumequinum* can emit high frequency ultrasonic calls (82kHz), whereas species like *N. noctula* can have low frequency calls within the sonic range (18kHz), other species emit frequency modulated sweeps (e.g. *Myotis spp*.), constant frequency calls (*R. ferrumquinum*) and calls combining frequency modulation and constant frequency elements (e.g. *Pipistrellus* spp.) (Russ 2012). The results suggest that the response of bats to traffic noise is a generalised phenomenon that has a negative impact across all functional groups examined. It is notable that sound in the sonic spectrum had a negative impact on the activity of all species, whereas ultrasound produced less marked responses and was absent in some species. This suggests that the mode of action is likely to be through general deterrence and avoidance, rather than through the masking of echolocation calls used for

orientation or foraging. These results are similar to the findings of laboratory based studies (e.g. Luo, Siemers & Koselj 2015). Comparable results were also identified from perceived point noise sources, that caused acoustically-mediated distractions for bats (e.g. Bunkley & Barber 2015). In addition, high frequency sound waves (ultrasound) propagate over smaller distances through air than lower frequency sounds. Therefore, the effective distance over which ultrasound generates an ecological impact — whether derived from experimental playback or from real roads — is likely to be lower than for sonic noise. It is unlikely that our speakers fully replicated the true coverage and extent of real road noise. However, this means that the effects observed in this study are likely to be conservative estimates of the true impact of traffic noise on free living bat species.

The deterrent effects of traffic noise, within the local area of the Experimental locations, were evident at distances of at least 20m from the source in our experiment, and was more severe beside the sources of the noise and in the direction it is coming from. Nevertheless, effects are still observed behind the speakers because noise drifts and bounces off objects, emphasising that consideration must be given to the diffusion of noise through space. Further work is required to establish the spatial scale of the impacts. This is important as many studies have demonstrated a reduction in general animal species diversity and abundance in relation to distances to roads (Benítez-López, Alkemade & Verweij 2010; Berthinussen & Altringham 2012b; Wiącek *et al.* 2015; Claireau *et al.* 2019b); our results suggest that traffic noise is likely to be an important factor. Nevertheless, other studies do demonstrate that fast flying bats are not as affected by roads and traffic noise compared to slower flying bats though (Bonsen, Law & Ramp 2015; Myczko *et al.* 2017). It is unclear however, whether bats would habituate to playback noise over the long-term, but over the relatively short duration of our study, the aversive effects appeared too consistently low over time.

Traffic noise reduced feeding activity (measured as the ratio of feeding calls to orientation calls) for both *P. pipistrellus* and *P. pygmaeus* — species with very similar echolocation patterns. When exposed to sonic and ultrasonic noise separately, stronger negative effects were observed for the former. The results of these experiments therefore support the inferences made by previous contrasting studies: ultrasonic noise reduces bat ability to feed, potentially by masking the echolocation calls used by foraging bats (e.g. Schaub, Ostwald & Siemers 2008; Siemers & Schaub 2011), but there is also a larger effect from sonic noise which does not overlap with the echolocation calls (Luo, Siemers & Koselj 2015). The mechanism for the latter is unclear but it may appear that the foraging bats actively avoid the aversive stimulus of traffic noise.

Now that we have established that the experiments have shown significant negative impact of traffic noise on bat activity and foraging behaviour using a single sound file, future research should focus on examining the effects of multiple target sound files (traffic noise) with similar components recorded over longer periods of time (Kroodsma *et al.* 2001). Additionally, a 'cross playback' (reversing the control and treatment locations after the initial experiment) could be conducted to ensure that all local variation within sites are considered and accounted for within the experimental design.

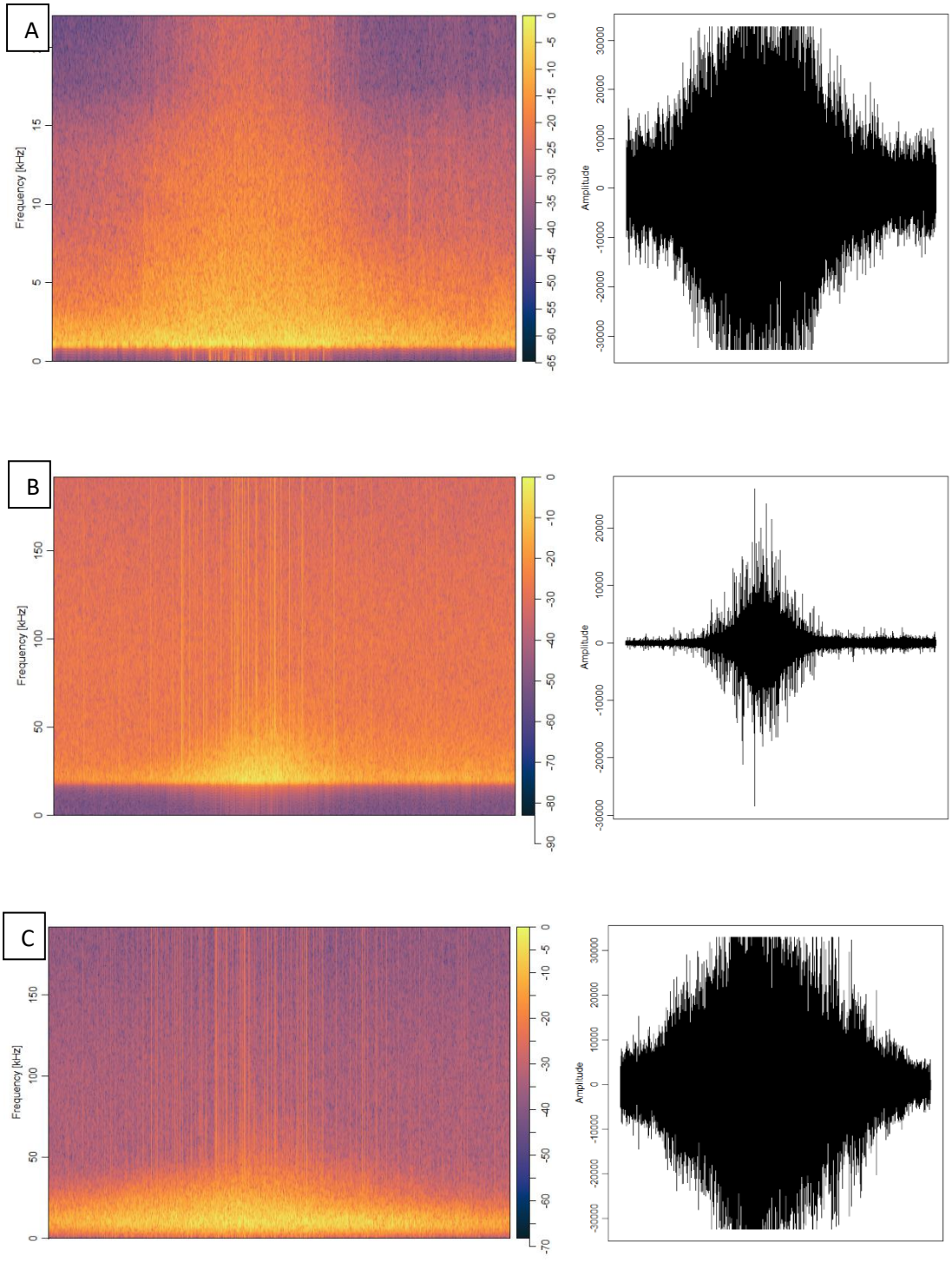
Many bats are of high conservation concern (e.g. in Europe, all species are protected under the Habitats Directive 92/43/EEC). Environmental Impact Assessments therefore need to consider the potential effects of road noise on habitat quality, landscape connectivity, and population viability. These effects need to be considered in combination to those of street lighting, collision and direct habitat loss and prioritised accordingly (e.g. Stone, Jones & Harris 2009; Day *et al.* 2015; Mathews *et al.* 2015; Azam *et al.* 2018; Pauwels *et al.* 2019). Given that road noise increases with the extent of traffic flow, the ecological impacts of greater traffic flow on existing routes — whether generated by transport policies or by specific projects such as a peri-rural housing development — must be considered, not just new road construction schemes. Potential

mitigation strategies include noise barriers, substrate alterations and speed limits (Wayson 1998; Ishizuka & Fujiwara 2004) but research is needed to test the effectiveness and proportionality of alternative strategies. New mitigation strategies are particularly needed to reduce the impact of sonic noise created by vehicles. Unfortunately, this is more difficult than mitigation for ultrasound that is readily attenuated over a short distance in air. Although the transition to electric vehicles may reduce road noise within urban centres, it is unlikely to have a material impact for most roads because at speeds >75km/h, sound is generated primarily by the contact between the tyres and road surface rather than by engines (The Highway Agency *et al.* 2011). Alterations to tyre composition and structures are therefore a more promising route to reducing traffic noise.

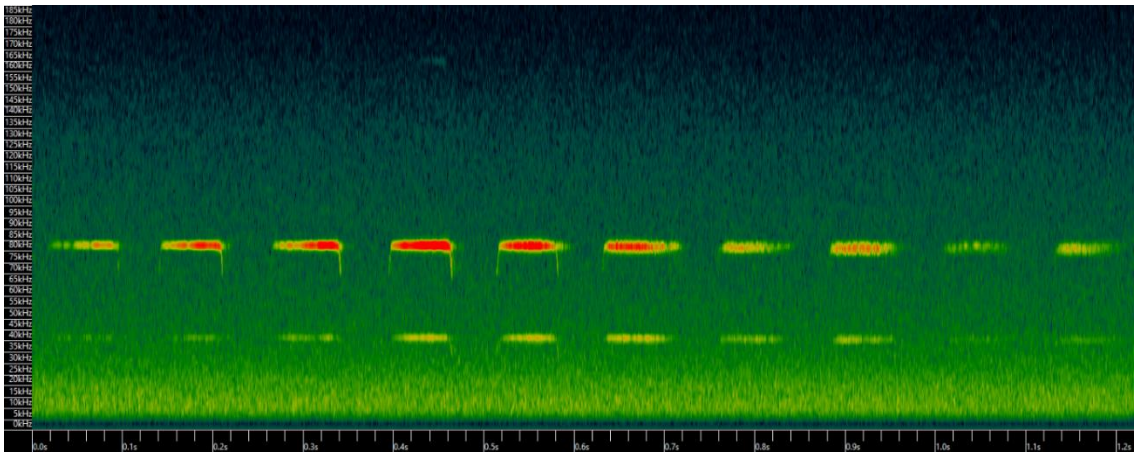
2.6. Acknowledgements

We thank Flavio Winkler for assistance with fieldwork and sound analysis, site owners for allowing access, and Anna David and Mike Symes from the Devon Wildlife Trust for obtaining landowner permissions. This research is supported by NERC (NE/S006486/1) and a PhD studentship funded by the Vincent Wildlife Trust, the Devon Wildlife Trust, the University of Exeter, and the University of Sussex.

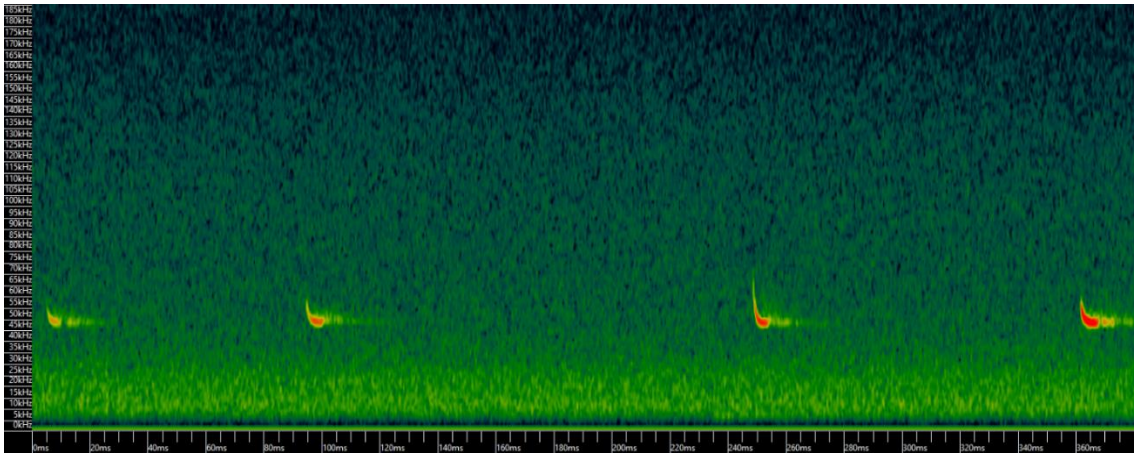
2.7. Appendices



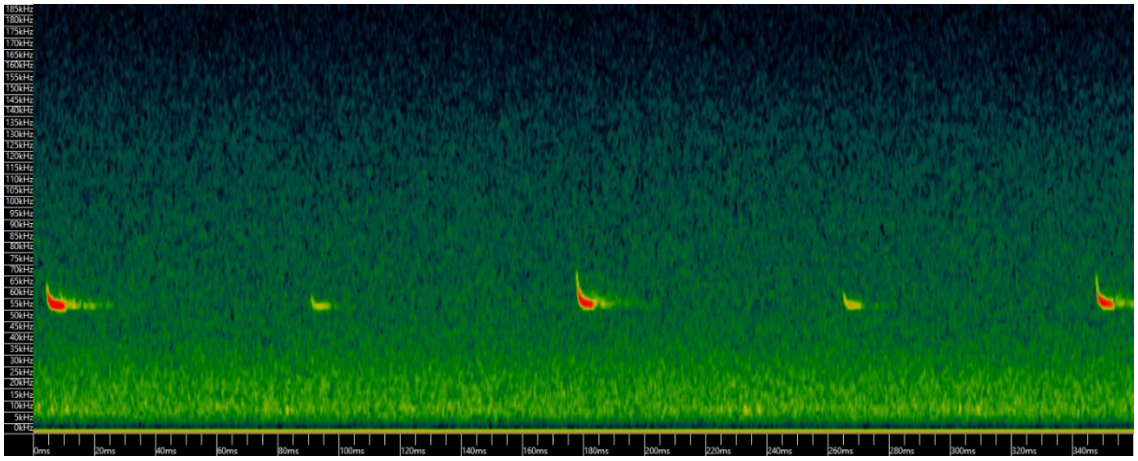
Appendix 2.7.1 Spectrogram and power spectrum of 1.4 second traffic noise playback clip used during the experiment. Sonic (A), ultrasonic (B) and both sonic/ultrasonic combined (C).



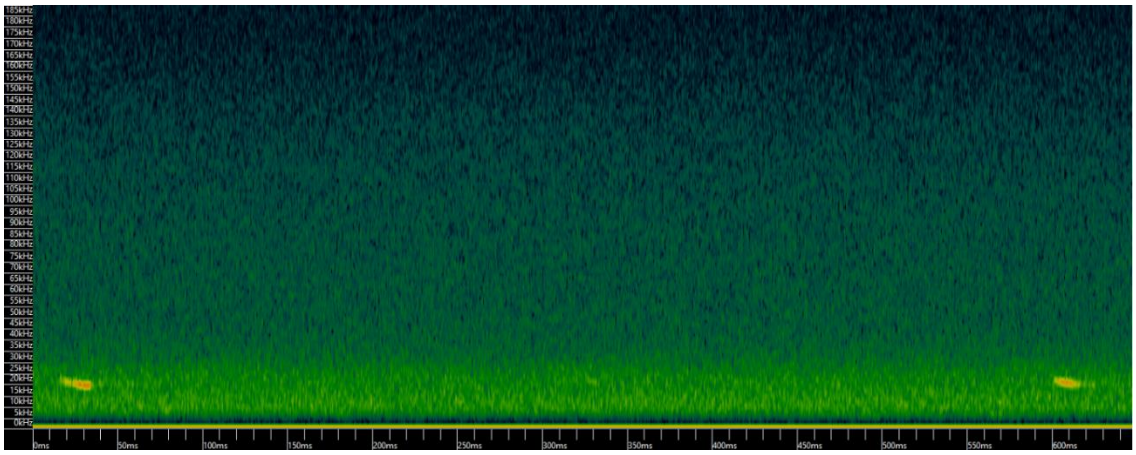
Appendix 2.7.2: Example of a greater horseshoe bat call (*Rhinolophus ferrumequinum*). Time in seconds is on the x-axis and frequency in kHz is on the y-axis.



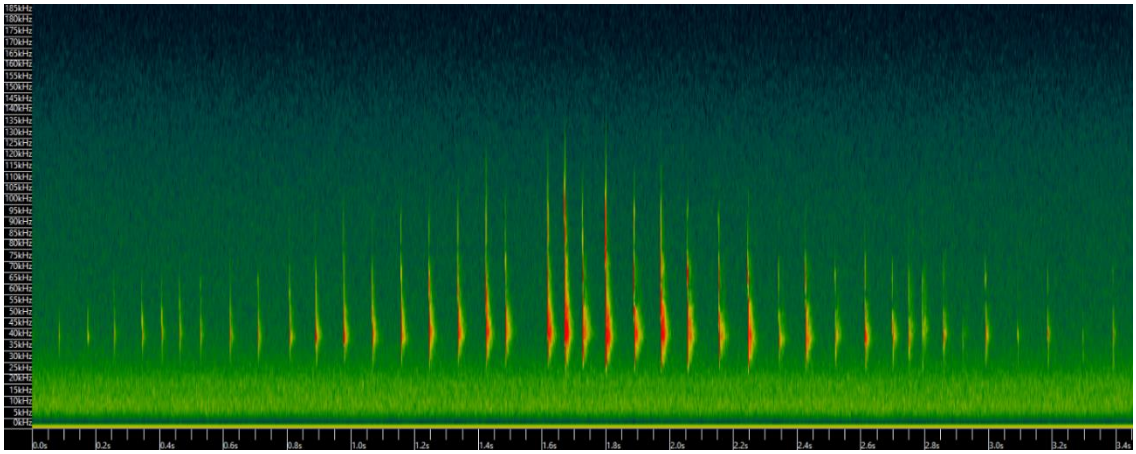
Appendix 2.7.3: Example of a common pipistrelle bat call (*Pipistrellus pipistrellus*). Time in milliseconds is on the x-axis and frequency in kHz is on the y-axis.



Appendix 2.7.4: Example of a soprano pipistrelle bat call (*Pipistrellus pygmaeus*). Time in milliseconds is on the x-axis and frequency in kHz is on the y-axis.



Appendix 2.7.5: Example of a noctule bat call (*Nyctalus noctula*). Time in milliseconds is on the x-axis and frequency in kHz is on the y-axis.



Appendix 2.7.6: Example of a *Myotis* bat call. Time in seconds is on the x-axis and frequency in kHz is on the y-axis.

Species	Location	Control			Combined sonic and ultrasonic traffic noise playback		
		Total	Mean	SD	Total	Mean	SD
Total bat activity	Control	1265	115	144	1697	100	107.1
	Experimental	10462	255.2	358	3407	58.7	70.9
Rhinolophus ferrumequinum	Control	163	14.8	22.1	273	16.1	24.2
	Experimental	178	4.3	5.5	164	2.8	7.2
Pipistrellus pipistrellus	Control	538	49	76.4	531	31.2	37.4
	Experimental	2933	71.5	89.2	1247	21.5	40.3
Pipistrellus pygmaeus	Control	442	40.2	54.6	723	42.5	53.4
	Experimental	4187	102.1	180	480	8.3	13.4
Nyctalus noctula	Control	17	1.7	3.8	47	2.8	3.4
	Experimental	916	22.3	63.7	725	12.5	32
Myotis species	Control	90	8.2	7.6	105	6.2	8
	Experimental	1931	47.1	99.6	634	11	31

Appendix 2.7.7**:** Data showing total, mean and standard deviation (SD) of species activity recorded during control nights and nights when traffic noise were being played at both control and experimental locations.

Species	Location	Control			Sonic traffic noise playback			Ultrasonic traffic noise playback		
		Total	Mean	SD	Total	Mean	SD	Total	Mean	SD
Total bat Activity	Control	342	85.5	92.2	667	66.7	90.9	550	55	72.4
	Experimental	5112	222.3	321.9	2539	56.4	67	4840	105.2	138.9
Rhinolophus ferrumequinum	Control	0	0	0	2	0.2	0.6	11	1.1	2.6
	Experimental	108	4.7	15.7	80	1.8	6.8	235	5.1	19
Pipistrellus pipistrellus	Control	203	50.8	78.9	486	48.6	73.3	314	31.4	51.8
	Experimental	2441	106.1	156.8	1283	28.5	35.8	2391	52	61.9
Pipistrellus pygmaeus	Control	31	7.8	11.1	39	3.9	6.2	76	7.6	14.4
	Experimental	760	33	77.6	610	13.6	34.8	1212	26.3	60.1
Nyctalus noctula	Control	27	6.8	13.5	13	1.3	3	63	6.3	12.9
	Experimental	13	0.6	1.6	2	0.04	0.2	23	0.5	1.7
Myotis species	Control	1	0.3	0.5	0	0	0	3	0.3	0.7
	Experimental	47	2	2.2	33	0.7	1.2	76	1.7	2.2

Appendix 2.7.8**:** Data showing total, mean and standard deviation (SD) of species activity recorded during control nights and nights when both sonic and ultrasonic traffic noise were being played at both control and experimental locations.

Chapter 3

Habitat associations of bats in an agricultural landscape: linear features versus open habitats.

3.1. Abstract

Bat species are important within agricultural landscapes, providing a number of different ecosystem services e.g. pest control and pollination. Yet, agricultural intensification is considered a leading cause of biodiversity loss and fragmentation at a landscape scale. However, it is not known the extent at which bats use linear features when foraging and commuting in agricultural settings, when compared to the interior of fields – which is not generally monitored during Environmental Impact Assessments. As part of a large-scale citizen science project, bat detectors were placed at 24 paired locations to examine whether activity differed between the centre of agricultural fields and the linear features (hedgerows and treelines) immediately surrounding them. The relationship between bat activity and individual linear feature types were then examined at 106 locations to determine which of the three categories (intensively managed hedgerows, sympathetically managed hedgerows and treelines) were utilised by bats in an agricultural landscape. Our results show that all 10 of the bat species found along linear features in our study also occurred in the middle of agricultural fields. Of the five species groups with sufficient data to examine separately, all had significantly more bat activity along linear features compared to the middle of fields, except for *Nyctalus noctula*. However, our results did show that 29% of calls from *Rhinolophus ferrumequinum* — a species generally considered to be highly restricted to linear features — were registered in the middle of agricultural fields; whereas this only accounted for 10% of *Pipistrellus pipistrellus* activity. Bat species were more likely to be associated with treeline compared to any other linear feature type, with no species were found to be significantly associated with intensively managed hedgerows (< 2m high). Our results highlight the importance of linear feature management to bat conservation.

3.2. Introduction

Agricultural intensification is considered one of the most important drivers of global declines in biodiversity, through habitat loss, transformation and fragmentation (Foley *et al.* 2011). In the past agricultural landscapes were structurally heterogeneous, consisting of a myriad of agricultural and semi-natural habitats in close proximity, offering relatively favourable habitat for wildlife (e.g. Weibull, Bengtsson & Nohlgren 2000). However, over the last 100 years, agricultural land has become more homogeneous, with increased land parcel sizes having high negative impacts on wildlife (Robinson & Sutherland 2002; Benton, Vickery & Wilson 2003). To facilitate this increase in parcel size many historical linear features (LF) were removed altogether from Europe's landscapes, in some cases as much as 71% (Sklenicka *et al.* 2009).

Treelines and hedgerows play a crucial role for biodiversity by providing structural heterogeneity, foraging grounds, breeding habitat and functional connectivity in the wider landscape for numerous taxa from small mammals and bats (Gelling, Macdonald & Mathews 2007; Kelm *et al.* 2014), to birds (Hinsley & Bellamy 2000) and invertebrates (Dover & Sparks 2000). Additionally, LF provide ecological services such as reducing soil erosion, increasing water retention and reducing pest incidences, as well as providing food and shelter for farm livestock (Baudry, Bunce & Burel 2000). Despite the historic loss, unfavourable management and neglect of hedgerows and treelines, their ecological importance is recognised and they are a priority habitat across Europe; financial incentives are provided for their conservation and management through Agri-Environmental Schemes (AES; Boughey *et al.* 2011).

Many European bat species are highly associated with LF (e.g. Walsh & Harris 1996b; Verboom & Spoelstra 1999). These features have been shown to increase functional connectivity and permeability into the environment at a landscape scale, thus reducing the 'barrier effect' caused by other features such as streetlights and roads (Stone, Jones & Harris 2009; Berthinussen & Altringham 2012b; Finch *et al.* 2020). There is, however, a tendency to dismiss the potential importance of open habitats, compared to LF, for species other than Nyctalus and Eptesicus bats

(e.g. Verboom & Huitema 1997) without quantifying relative amounts of activity in each habitat type. Recent research illustrated the importance of cattle-grazed fields, with both *Rhinolophus ferrumequinum* and *Pipistrellus pipistrellus* showing significantly more activity there compared to un-grazed fields (Ancillotto *et al.* 2017). These results highlight the relative importance of certain open agricultural habitats for bats, regardless of the amount of edge habitat or natural vegetation surrounding the site.

Here, we compare both occurrence and relative activity of bat communities found along LF and in open agricultural fields (both arable and pasture; all pasture fields apart from one, which had sheep, did not have livestock in them). In addition, we compared different types LF, sympathetically managed hedgerows, intensively managed hedgerows, and treelines, to assess their relative importance for British bat species. We also discuss the possibility of how historical LF might influence the abundance of species records found in present day open agricultural fields using the ROAM database (http://digimap.edina.ac.uk/roam/historic).

3.3. Methods

3.3.1. Site selection

Sites were selected at four *R. ferrumequinum* roost sustenance zones (RSZ: area of land within 4 km of a roost) in Devon, England. Detectors were placed in a paired design (n=24) to examine the extent to which bat communities use open agricultural fields verses to the LF immediately surrounding them. To determine how bats utilised different LF types within an agricultural setting, 106 sites were selected; intensively managed hedgerows (n=17), sympathetically managed hedgerows (n=45) and treelines (n=44). These were chosen based on the approximate habitat availability of these features within the landscape and access to them. LF's were defined as follows: intensively managed hedgerows are those hedgerows typically cut annually and which have a median height < 2m, sympathetically managed hedgerows are those with a median height > 2m that have not been cut in the previous year, and treelines are defined as those sympathetically managed hedgerows > 6m that have trees along the length of the feature.

3.3.2. Acoustic surveys

Bat activity data were collected between the 26th July and the 11th September 2016, as part of a large citizen science project (Devon Greater Horseshoe Bat Project). Volunteers were asked to place full spectrum bat detectors (SM2 and SM2+ detectors with an SMX-U1 and SMX-US ultrasonic microphone, Wildlife Acoustics, Maynard, Massachusetts, USA) at the survey locations which had been previously identified as suitable. All detectors were pre-set to the manufacturer's specifications before being placed out by the volunteers. Details of the acoustic detector settings are provided in Appendix 3.7.1. Microphones were placed at a height of at least 1 m off the ground and were orientated horizontally. The detectors were set to record from 30 minutes before sunset to 30 minutes after sunrise, for a period of up to seven nights.

All bat passes were analysed using Kaleidoscope Pro software (version 3.1.1; Bats of Europe classifier version 3.0.0; Wildlife Acoustics, Maynard, Massachusetts) and were verified manually on the basis of call frequency, shape and repetition rate. Bat passes were defined as one or more echolocation call within one second of each other (Fenton 1970; Walsh & Harris 1996a). Detailed characteristics of the evolutionary traits of all species examined in this study, including their call structure and their foraging strategies, can be found in Russ (2012) and Dietz and Kiefer (2016).

3.3.3. Statistical analysis

Statistical analyses were undertaken using R (version 3.3.0; R Core Team 2016). To investigate the relationship between bat activity and habitat type, generalised linear mixed models with a negative binomial distribution were built for each species using the 'lme4' package (Bates *et al.* 2015). When examining the relationship between LF and the centre of agricultural fields; RSZ (two zones), site (unique field ID) and individual detector identities were set as random factors to account for the potential non-independence of data gathered on consecutive nights. Detector identity was nested within site, to account for the paired structure of the study. Those detectors placed in the middle of the agricultural fields were then extracted to create a subset dataset and a new model. Total bat species activity was used as the predictor variable, with distance to LF,

field type (arable or pasture field) and whether historical LF were present or not in the past (ROAM database) were all used as fixed effects in the model. RSZ were used as a random effect.

To assess the relative effects individual LF had on species activity, LF type were used as predictor variables, with individual detector identities included as a random factor. RSZ (four zones) was also used as a random factor for all species groups apart from *R. ferrumequinum*, which it was used as a fixed factor. This was done to test whether the data collected at the four roost locations significantly influence bat activity. If interactions were identified to be significant, multi-comparison Tukey adjusted post-hoc tests were conducted using the 'lsmeans' package (Lenth 2016); to test for pairwise differences between levels of the relevant factors.

3.4. Results

Nine species of bat were individually identified: *Barbastella barbastellus*, *Eptesicus serotinus*, *Nyctalus noctula*, *Pipistrellus nathusii*, *P. pipistrellus*, *Pipistrellus pygmaeus*, *Plecotus auritus*, *Rhinolophus hipposideros, R. ferrumequinum,* and the genus *Myotis*. All species recorded occurred both in the middle of fields and along LF. Sufficient data were only available to examine relative activity of five species groups: Total species (all species combined), *R. ferrumequinum, P. pipistrellus, P. pygmaeus* and *N. noctula*.

Four of the species groups investigated had higher bat activity at the LF compared to the middle of field (Table 3.4.1): *R. ferrumequinum* (Odds Ratio (OR): 3.51, CI: 1.90 – 6.47, $p < 0.001$), *P. pipistrellus* (OR: 7.14, CI: 4.35 – 11.42, $p < 0.001$), *P. pygmaeus* (OR: 7.0, CI: 3.49 – 14.01, $p < 0.001$), as well as total activity of all species recorded (OR: 4.11, CI: 2.74 – 6.16, $p < 0.001$). *N. noctula* was the only species not to illustrate a significant difference (OR: 1.10, CI: 0.69 – 1.74, $p = 0.69$). Using the subset of data consisting of only records in the middle of agricultural field, we highlight that distance to LF (median: 76, range: 30 – 147), field type and the presence of historical ROAM LF did not significantly influence total bat activity (all *p*-values > 0.05).

There were significant interactions between LF type and the relative activity for *R. ferrumequinum* (p = 0.014) and *P. pygmaeus* (p < 0.001). However, LF type did not affect *P. pipistrellus* (interaction term: p = 0.26), *N. noctula* (interaction term: p = 0.25) or total bat activity (interaction term: p = 0.16). Using a post-hoc test, intensively managed hedgerows were found to have a significant negative effect on for *R. ferrumequinum* (χ^2 = -0.91, p = 0.04) and *P. pygmaeus* (χ^2 = -1.38, p = 0.006) activity when compared to treelines. Similarly, significant higher activity was recorded along treelines when compared to sympathetically managed hedgerow for *P. pygmaeus* (χ^2 = 0.81, p = 0.02). There was no significant difference between *R. ferrumequinum* activity at sympathetically managed hedgerows compared with either treelines (χ^2 = 0.05, p = 0.98) or intensively managed hedgerows (χ^2 = 0.86, p = 0.06; Table 3.4.2).

The interaction between RSZ and *R. ferrumequinum* activity was also significant (p = 0.005), with less activity recorded at the RSZ that had the smallest roost compared to in two RSZ that had larger colony sizes (χ^2 = -1.32, p = 0.01; χ^2 = -1.41, p < 0.001).

Table 3.4.1: Species activity found along linear feature compared to in the middle of agricultural fields.

Species or genus	Total No. of passes recorded		Mean No. of passes per night, SD and the percentage					
	Field	Linear Feature	Field			Linear Feature		
			Mean	SD	%	Mean	SD	%
Rhinolophus ferrumequinum	225	396	1.6	3.5	29	3.9	4.4	71
Pipistrellus pipistrellus	482	3214	3.4	5.4	10	31.5	54.8	90
Pipistrellus pygmaeus	244	1036	1.7	3.9	15	10.2	19.3	85
Nyctalus noctula	536	419	3.8	8.3	48	4.	9.2	52
Total species	1796	5679	12.8	13.6	19	55.7	67.9	81

Table 3.4.2: A comparison between species activity recorded at the three different types of linear features: intensively managed hedgerows (IM), sympathetically managed hedgerows (SM) and treelines (T).

Species or genus	Total No. of passes recorded			Mean No. of passes per night, SD and the percentage								
	IM	SM	T	IM			SM			T		
				Mean	SD	%	Mean	SD	%	Mean	SD	%
Rhinolophus ferrumequinum	618	1174	3268	5.8	10.4	15	5.0	8.3	14	26.8	80.8	71
Pipistrellus pipistrellus	3074	13304	7923	28.7	47.6	19	56.4	137.6	38	64.9	103.4	43
Pipistrellus pygmaeus	443	2511	9597	4.1	11.9	5	10.6	21.5	11	78.7	297.1	84
Nyctalus noctula	293	931	421	2.7	8.7	27	3.9	8.8	38	3.5	6.2	35
Total species	5724	20846	26370	53.5	66.6	15	88.3	55.4	25	216.1	499.2	60

3.5. Discussion

This research demonstrates the important relationship LF have on bat activity in an agricultural landscape, and illustrates the need for their protection and appropriate management. Four of the five species groups analysed showed significantly higher bat activity at LFs compared with the middle of fields. However, the frequency at which species were recorded in the middle of fields differed. Our results are mirrored in other studies which report open habitats having less bat activity (e.g. Walsh & Harris 1996b; Verboom & Huitema 1997; Boughey *et al.* 2011; Kelm *et al.* 2014). The attraction of bats to LF can largely explained by higher food availability, protection from predators and wind (Verboom & Spoelstra 1999; Downs & Racey 2006), and the use of LF for navigation (Schaub & Schnitzler 2007).

Out of those species significantly associated with LF, we identify that almost a third, 29%, of *R. ferrumequinum* activity is recorded in the centre of agricultural fields. Such findings are unusual for species which tend to be heavily associated with LF or woodland (e.g. Billington 2008). This highlights the importance of such areas when designing acoustic surveys and the need to include 'sub-optimal' habitat into Environmental Impact Assessments; to get an accurate understanding of how bats are utilising the landscape throughout the year.

Using the ROAM database, we assessed whether the presence of bats in the middle of fields could be due to a historical legacy of hedgerows being present in those locations in the past, acting as old commuting routes and foraging grounds for the bats. This, however, did not seem to influence the results, with the highest nightly *R. ferrumequinum* and total species activity being recorded in fields which did not have any previous LF within them since the earliest ROAM records for these locations in the 1930s. In addition, whilst we could not examine the effect of seasonality as part of this study owing to its methodological design, we anecdotally record higher bat activity in the middle of fields later in the season (September). This is in line with the results obtained by Kelm *et al.* (2014), who demonstrated that LF had weaker effects on bat

activity later on in the season (July-October). This could be because more bats are present post breeding, leading to higher food demands, as well as the seasonality of prey items.

Overall, our findings show that two of species groups had significantly more activity recorded along treelines compared to hedgerows. This reiterates the results from Wickramasinghe *et al.* (2003), Brandt *et al.* (2007) and Froidevaux *et al.* (2019), who found that increased bat activity and foraging potential occurred along LF on agricultural land which had trees and taller shrubs present. This is especially true for bat species associated with woodlands and woodland-edge habitat (Russ & Montgomery 2002; Nicholls & Racey 2006; Fuentes-Montemayor *et al.* 2013). Boughey *et al.* (2011) found that unlike height, hedgerow width has not been found to influence bat activity, but the length of individual hedgerows and the total length at which they occur in the surrounding landscape are important for increased activity (Verboom & Huitema 1997). It is not only bats that are significantly associated with well-developed treelines and taller hedgerows. They have been shown to increase floral diversity, as well as moth species richness and bird abundance/density (MacDonald & Johnson 1995; Merckx *et al.* 2012; Staley *et al.* 2012; Froidevaux, Broyles & Jones 2019). Similarly to bats, they can act as food stores, shelter belts (creating microclimates) and breeding/roosting locations for other species (Hinsley & Bellamy 2000; Maudsley 2000; Staley *et al.* 2012). However, these increase in diversity along LF critically comes down to their management, with those that are cut every three years showing some of the highest benefit for biodiversity (Staley *et al.* 2012; Froidevaux *et al.* 2019). Staley *et al.* (2012) shows that by reducing cutting frequency from every year to every 3 years resulted in 2.1 times more flowers and a 3.4 times greater berry mass over 5 years. These associations and benefits may be due to the structural changes in LF as they develop and succeed from sympathetically managed hedgerows into treelines (MacDonald & Johnson 1995).

Due to their significant importance to a wide variety of species, LF are key landscape feature for the movement of wildlife in fragmented environment, yet they are under threat. The total length

of LF has decreased by approximately 23% over 16 years (Barr & Gillespie 2000) and the number of trees present within them has also reduced by 6.6% over a 20 year period (Carey *et al.* 2008). Boughey *et al.* (2011) speculates that this is due to management regimes, illustrating the point that new trees in hedgerows add cost to mechanical trimming and decrease crop production through shading. However, as our results show, increased LF height showed greater bat activity, having the potential to act as critical corridors and foraging areas for the long-term survival of populations. Such corridors provide even more important resources during periods of lactation, as females tend to travel shorter distances to forage; increased travel time to foraging grounds can negatively impact juvenile growth and survivorship (Clark, Leslie Jr & Carter 1993; Kerth & Melber 2009).

Appropriately managing, retaining and rebuilding the countryside's network of LF, particularly those of higher quality (sympathetically managed hedgerows and treelines), through result based financial incentives from AES (or otherwise) is vital to the conservation of bats and the many other species using them (Froidevaux *et al.* 2019; Froidevaux, Broyles & Jones 2019). Appropriate ecological assessments and considerations of cumulative impacts at a landscape scale need to be given when examining the effects of agricultural practices.

3.6. Acknowledgements

We would like to thank all of the citizen scientists and landowners who help with the research, and Anna David and Mike Symes who helped in obtaining land-owner permissions. We thank Patrick G. R. Wright for his comments on the manuscript. This research is a PhD studentship funded by the Vincent Wildlife Trust, the Devon Wildlife Trust, the University of Exeter, and the University of Sussex.

3.7. Appendices

Appendix 3.7.1: Detector settings used for both the SMX-U1 and SMX-US microphones used in conjunction with SM2 and SM2 bat+ detectors (Wildlife Acoustics, USA) during the acoustic bat surveys.

Detector settings	SMX-U1	SMX-US
Sampling rate	192000 kHz	192000 kHz
Gain	12dB	48dB
High pass filter	4 kHz	4 kHz
Low pass filter	Off	Off
Trigger level	18 SNR	18 SNR
Trigger window	2.0 sec	2.0 sec

Chapter 4

Modelling the functional connectivity of landscapes for greater horseshoe bats *Rhinolophus ferrumequinum* at a local scale.

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4.1. Abstract

The importance of habitat connectivity for wildlife is widely recognised. However, assessing the movement of species tends to rely on radio-tracking or GPS evidence, which is difficult and costly to gather. To examine functional connectivity of greater horseshoe bats (*Rhinolophus ferrumequinum*) at a local scale using Circuitscape software; comparing our results against expert opinion 'fly ways'. Expert opinions were used to rank and score five environmental layers influencing *R. ferrumequinum* movement, generating resistance scores. The slope and resistance scores of these layers were varied, and validated against independent ground truthed *R. ferrumequinum* activity data, until a unimodal peak in correlation was identified for each layer. The layers were combined into a multivariate model and re-evaluated. Radio-tracking studies were used to further validate the model, and the transferability was tested at other roost locations. Functional connectivity models could be created using bat activity data. Models had the ability to be transferred between roost locations, although site-specific validation is strongly recommended. For all other bat species recorded, markedly more (125%) bat passes occurred in the top quartile of functional connectivity compared to any of the lower three quartiles. The model predictions identify areas of key conservation importance to habitat connectivity for *R. ferrumequinum* that are not recognised by expert opinion. By highlighting landscape features that act as barriers to movement, this approach can be used by decision-makers as a tool to inform local management strategies.

4.2. Introduction

Retaining the functional connectivity of landscapes is a pressing issue for conservation (Goodwin & Fahrig 2002; Fahrig *et al.* 2011). Largely driven by urbanisation and agricultural change, increasing habitat fragmentation has implications at an individual and population level. The consequences include isolation from habitats necessary for foraging, resting or gene flow, resulting in population declines and greater vulnerability to extinction (Pulliam 1988; Beier 1993; Rossiter *et al.* 2000).

The identification of landscapes or habitats that provide high functional connectivity for species of conservation concern has the potential to focus resources where they can be deployed most effectively (Lawton 2010). For some species, such habitats are —at least in principle — legally protected because they are vital to maintaining the integrity of key populations (e.g. landscapes connecting a network of Special Areas of Conservation of bats under the EU Habitats Directive; 92/43/EEC (EC 1992)). However, in practical terms, trying to identify the exact locations or the extent of these habitats can be extremely challenging, with many habitat requirements being species specific (Fagan & Calabrese 2006; Fahrig 2007). For example, important corridors may offer relatively poor habitat quality in themselves, but may offer the best — or only — available route to join areas important for foraging, mating or resting.

One approach to exploring and visualising functional connectivity within a landscape is to use circuit theory (McRae 2006). In combination with random walk theory (Doyle & Snell 1984; Chandra *et al.* 1996), these approaches allow for all available movement possibilities to be considered and mapped using resistance surfaces. These surfaces (landscapes) are scored based on the cost incurred for an individual to move between two nodes (habitats) (Wiens 2001), with less resistance representing an increased probability of movement between nodes. Linking nodes together creates cost paths that can be represented by a cumulative resistance value or cost-weighted distance (McRae *et al.* 2008). Thus, the probability of movement between any two spatial locations can be measured, whilst considering all other available routes.

The application of this approach, using the software Circuitscape (McRae *et al.* 2008), has been successfully used to map barriers to gene flow and species movement, and to identify landscape corridors critical to the long term viability and stability of populations (Belisle 2005; e.g. Rayfield *et al.* 2016; Le Roux *et al.* 2017). However, most of this research has focused on large spatial scales (e.g. country-level), and has used direct measures of animal movement (e.g. GPS tracks). In practice, barriers to connectivity, as well as conservation actions, frequently operate at much smaller spatial scales. For example, decisions must be made about the probable effect of a single, lane major road on the ability of a local population to access parts of its habitat, and hence what, if any, mitigation is required.

Considering the cost implications and the lack of equipment to be able to GPS smaller bat species safely and ethically, we highlight the need to be able to develop non-invasive methods for examining conservation issues surrounding landscape fragmentation at a local scale. This is of particular concern for the greater horseshoe bat (*Rhinolophus ferrumequinum*) which has suffered large worldwide declines and is of particular conservation concern in Britain (Jones *et al.* 2009). This species is highly dependent on linear features, such as hedgerows, to facilitate movement into the wider landscape (Duvergé & Jones 1994; Froidevaux *et al.* 2017). Using an approached detailed by Shirk *et al.* (2010), we use *R. ferrumequinum* in southern Britain to test whether i) robust, high resolution connectivity models suitable for informing conservation planning at local scales can be produced using Circuitscape, ii) non-invasive indicators of activity can be used to populate models of functional connectivity, and iii) the optimal connectivity model output corresponds with expert opinion 'fly ways'.

4.3. Methods

4.3.1. Study area and GIS data

The study areas were defined as 3km radii around four *R. ferrumequinum* maternity roosts in Devon, southwest England (Appendix 4.7.3). These study areas were restricted to 3km due to computational limitations regarding the trade-off between the extent of the area covered and

the resolution of the data. As *R. ferrumequinum* are site-faithful (Rossiter *et al.* 2002), with limited movement of females between sites during the maternity season, the data collected from these roosts were treated as independent from each other during the modelling process. In addition, the roosts were between 13.5km – 89km apart. The maximum distance recorded by an individual during our radio telemetry study was 9.1km (mean: 5.4km); this is in line with Pinaud *et al.* (2018), who recorded a maximum distance of 7.6km (mean: 4.2km). Each study area contained a mosaic of habitats and landscape features, including grazed and arable fields, broadleaved woodland, coniferous woodland, hedgerows, riparian habitats, and occasional farm buildings and residential houses (Appendix 4.7.4 – Appendix 4.7.7). Numerous single-lane roads crossed the landscape, and in two of the study areas there were two-lane highways. Immediately surrounding three of the roosts were small villages. Streetlights occurred in these villages, as well as in isolated patchy locations across the wider landscape.

One-metre resolution geographical information system (GIS) raster data were obtained for each landscape feature surrounding each of our roosts, resulting in five different environmental layers (Table 4.3.1). The *Lightscape* layers were created following the methodology described by Bennie *et al.* (2014), using streetlight position and height with Digital Terrain Models (DTM) and Digital Surface Models (DSM) to create a light irradiance GIS layer. These were used to predict the direction and intensity of streetlight at different wavelengths, modelling the night-time light environment. The *Distance to Roads* layers were created using ArcGIS and ranked using the most current annual average daily traffic volumes (AADT; rounded to the closest 10) (Department of Transport 2015). In this case, lower AADT meant a lower rank value. The *Distance to Linear Features* layers defined 'intensively managed hedgerows' as those typically cut annually and which have a median height < 2m; 'sympathetically managed hedgerows' are defined as those with a median height > 2m, that had not been cut the previous calendar year, and were managed, whether intentionally or not, in ways that benefit wildlife.

Table 4.3.1**:** GIS data used to model the movement of *Rhinolophus ferrumequinum* in the study site (average annual daily traffic – AADT).

Environmental layer	Landscape feature	Rank & AADT score	Reference
Land Cover	Orchards	Rank 1	EDINA (2016d)
	Deciduous woodland	Rank 2	Morton *et al.* (2011)
	Scrub	Rank 3	Morton *et al.* (2011)
	Grassland	Rank 4	Morton *et al.* (2011)
	Coniferous woodland	Rank 5	Morton *et al.* (2011)
	Arable land	Rank 6	Morton *et al.* (2011)
	Lake	Rank 7	Hughes *et al.* (2004)
	Buildings	Rank 8	EDINA (2016e)
Lightscape	GPS coordinates of lights, column height, light type	-	Devon and Cornwall County Council
	LiDAR – DSM	-	EDINA (2016a)
	LiDAR – DTM	-	EDINA (2016b)
Distance to River	River	-	EDINA (2016d)
Distance to Roads	Single lane local road	Rank 1 - AADT 660	EDINA (2016c)
	Single lane minor road	Rank 2 - AADT 3260	EDINA (2016c)
	Single lane major road	Rank 3 - AADT 15510	EDINA (2016c)
	Two-lane major Road	Rank 4 - AADT 41750	EDINA (2016c)
Distance to Linear Features	Sympathetically managed hedgerow	Rank 1	Broughton *et al.* (2017)
	Treeline	Rank 2	Broughton *et al.* (2017)
	Woodland edge	Rank 3	EDINA (2016d)
	Intensively managed hedgerow	Rank 4	Broughton *et al.* (2017)

4.3.2. Bat surveys

4.3.2.1. Acoustic surveys

The relative *R. ferrumequinum* activity was based on acoustic surveys for bats that were conducted as part of a citizen science project (Devon Greater Horseshoe Bat Project; June – September 2016). Acoustic data were collected at 205 survey points using full-spectrum static bat detectors (SM2 and SM2+ detectors with SMX-U1 or SMX-US ultrasonic microphones that were sensitivity-tested prior to deployment, Wildlife Acoustics, Maynard, Massachusetts, USA). Details of the acoustic detector settings are provided in Appendix 4.7.2. Microphones were

placed at a height of at least 1 m above the ground and were orientated horizontally. Recordings were made for up to seven nights from 30mins before sunset to 30mins after sunrise. Bat detectors were placed as close to randomly as possible (depending on landowner permission) in all available landscape features within 3km of each roost. During the process of univariate and multivariate model validation, no predictions within the peripheral 300m of the survey area were used, as it is anticipated that the validity of the model would decline at its outer extremities (Koen *et al.* 2010).

Acoustic records were analysed using Kaleidoscope software (version 3.1.1; Bats of Europe classifier version 3.0.0; Wildlife Acoustics, Maynard, Massachusetts, USA) and were verified manually on the basis of call frequency, shape and repetition rate. Relative bat activity was assessed as the average number of bat passes per night per detector during the survey period (e.g. Jung *et al.* 2012; Charbonnier *et al.* 2014). Any bat detectors that only functioned for a single night owing to malfunction, and that did not record *R. ferrumequinum* during that night, were excluded from further analysis. *R. ferrumequinum* passes were defined as pulses of sound, as described by Russ (2012), recorded within a single sound file. Sounds files were created by a rolling two-second window: once the detectors were triggered, recording continued until there was a two-second window without sound of sufficient amplitude to trigger recording. The average pass rate per night per detector were used to validate all models.

4.3.2.2. Radio tracking study

During May and June 2010 and 2012, 13 female *R. ferrumequinum* were caught using mist nets and harp traps for radio-tracking at Roost 2 in southern Devon, under licence from the National Statutory Nature Conservation Organisation (Natural England). Each bat was weighed, and the largest parous females were selected for study. The transmitter (0.35 g) did not exceed 5 % of the bat's body weight. A small area of fur was clipped from between the scapulae, and VHS radio-transmitters (Micro-pip, Biotrack Ltd., Wareham, Dorset, UK) were attached using Torbot surgical adhesive (Torbot Group Inc., Rhode Island, USA).

The female *R. ferrumequinum* were tracked nightly for up to ten days, or until the tags dropped off or their batteries failed. Bats were followed, as closely as possible without causing a disturbance, by, two teams of observers each equipped with radio receivers (Sika, Biotrack Ltd., Wareham, Dorset, UK) connected to hand-held directional three-element Yagi antennae; to establish commuting routes and foraging grounds *in situ* (White & Garrott 2012), fixes were taken every 5mins. Alternatively, the general locations of the bats were identified using an omni-directional magnetic whip aerial mounted on the roof of a vehicle. Once the teams homed in on the individual *R. ferrumequinum,* they switched to the hand-held equipment again, taking multiple timed bearings of the location of each bat. From these measurements, the position of the bats were then biangulated after each survey night. Using a similar approach, Pinaud *et al.* (2018) estimated the spatial accuracy to be approximately 100m. To eliminate temporal correlation of our fixes, each fix was considered independent when at least 30min separated two consecutive locations (White & Garrott 2012).

4.3.3. Modelling approach

An underlying premise of our approach was that relative *R. ferrumequinum* activity (in this case bat passes) are a suitable proxy for more direct indices of connectivity (e.g. genetic connectivity indices or animal movement tracks collected by GPS). Doncaster and Rondinini (2001); Braaker *et al.* (2014); Le Roux *et al.* (2017); and Pinaud *et al.* (2018) all demonstrate, through field observations, static bat detectors, radio-tracking and Geographical Positioning System (GPS) data, that in general species, including *R. ferrumequinum*, spend less time in unfavourable habitats that have higher resistance values. Additionally, the same individuals are more likely to occur multiple times, and at higher activity levels, in more favourable areas of low resistance values, e.g. along commuting routes or at foraging grounds (Doncaster & Rondinini 2001). To test this, we compared the outputs of our Circuitscape models with independent data gathered using both acoustic surveys and from radio-tracking studies at our study locations.

Landscape connectivity for *R. ferrumequinum* was hypothesized to be influenced by local-scale landscape heterogeneity. To make predictions on this hypothesis, we used a similar modelling framework to that outlined by Shirk *et al.* (2010), and expert opinion models were created as raster resistance surfaces (spatial models) for each environmental layer. Mathematical functions that varied resistance scores and slope values were applied (see below and Appendix 4.7.8), to the expert opinion model for each environmental layer, evaluating and identifying the peak relationship between the resistance surface parameters and the independent activity data collected around a single *R. ferrumequinum* roost (Roost 1). This process identified the optimal univariate models for each environmental layer. These optimal layers were combined into a multivariate model, which were then reanalysed to find the optimal multivariate model. In addition, we then compared the Circuitscape model output for Roost 2 against data collected through radio-tracking studies. To test the transferability of the multivariate model to other locations, we applied the same resistance values to the environmental layers at three other *R. ferrumequinum* roosts (Roosts 2–4); using independent ground truthed *R. ferrumequinum* activity data collected around each of those three roost locations to assess the utility of the models.

4.3.3.1. Expert opinion model

Based on eight expert opinions and a literature review of the movement and dispersal ability of *R. ferrumequinum* (Jones, Duvergé & Ransome 1995; Flanders & Jones 2009), 18 different landscape features were selected and ranked, within their respective environmental layer groups (Table 4.3.1), based on the likely resistance they posed to the movement of *R. ferrumequinum*. The experts were from both academic and non-governmental organizations, who specialise in, and have extensive knowledge of, *R. ferrumequinum* ecology. Each expert was sampled, via questionnaire, on the rank and potential resistance values of each landscape feature. These data were then combined to determine the initial ranks and resistance values. All experts were consistent in ranking the resistance on each landscape features. A rank of one

indicated the least costly landscape feature for the movement of *R. ferrumequinum*, while higher ranks were associated with more costly features. If there was only one landscape feature in a given environmental layer, then no ranks were required e.g. *Rivers*. However, if a layer had more than one landscape feature, e.g. *Roads*, then the maximum rank was the total number of features—in this case four; for other layers, such as *Land Cover*, the maximum was eight. Those landscape features with higher ranks have greater weighting associated with them, relative to others within the same layer, and as a result, they are more resistance to species movement. Both resistance, and subsequently cost surfaces, using expert opinion data, were then created for each of the environmental layers at Roost 1, before mathematical functions (see below) were applied and analysed during the univariate modelling process.

4.3.3.2. Mathematical functions

When examining an ecological system, the relationships between environmental layers (or their resistance values) and the functional response of the species (e.g. animal movement) are rarely linear (Etherington 2016). In addition, researchers do not often account for interactions between multiple environmental layers that can occur in real landscapes. For example, a hedgerow with and without streetlights on it will influence the movement of bat species in different ways (Stone, Jones & Harris 2009). To avoid these issues, and to maximise the potential accuracy of the models, we rescaled our raster data to permit a range of slope values (x; ranged from 1–5) relating to our resistance values. Additionally, we varied the maximum resistance value ($R_{max)}$, allowing for a range of resistance values to be considered for each layer (varied between resistance 1 and 10^{10}; see below and Appendix 4.7.8).

4.3.3.2.1. Land cover

The eight broad land cover features were ranked based on expert opinion in order of lowest to highest resistance (Table 4.3.1). The 'Buildings' landscape feature was always set as the lowest permeability. Resistance surfaces for *Land Cover* were created using the following equation:

$R = (Rank/V_{max})^{x}*R_{max}$

where R is the resistance for each raster pixel (each of which consist of a single *Land Cover* type) and V_{max} is a constant that is the highest possible rank for that feature type. For example, at three of our roost locations there were seven landscape features (Orchards, Deciduous woodland, Scrub, Grassland, Coniferous woodland, Arable land, Buildings; $V_{max} = 7$), and at one we had eight, because Lakes were only present for Roost 4 ($V_{max} = 8$). This means that as the expert opinion ranking moves nearer to the highest resistance rank (V_{max}), the overall resistance increases towards R_{max} at a rate controlled by the response curve of the slope value (x) (Shirk *et al.* 2010).

4.3.3.2.2. Lightscape

The lightscape irradiance (*IR*) values were multiplied by the slope values and maximum resistance:

$R = (IR)^{x}*R_{max}$

4.3.3.2.3. Distance layers

Each of the three continuous distance layer functions were modified in different ways based on their ecological relationship with *R. ferrumequinum*. Euclidean distance to *Rivers* was calculated using the following function:

$R = (D_{et}/V_{dmax})^{x}*R_{max}$

where D_{et} is the nearest distance of the raster pixel to any river in the 3km extent, and V_{dmax} is a constant that is defined as the maximum distance possible from *Rivers* within the extent of the 3km. Based on previous literature suggesting that *R. ferrumequinum* activity occurs at close proximity to linear features a maximum distance of 10m was set for both the *Linear Features* and *Rivers* layers (Ransome 1996).

Distances, to *Linear Features* were modelled in a similar way, except as there is more than one feature; the rank order of the features were based on the resistant values chosen by the expert opinion. The lower the expert opinion resistance value the higher the rank order of the feature, meaning that those variables with higher rank order are more permeable than those with a lower rank order. V_{rmax} is a constant representing the highest rank value for each layer, in this case four. Both the distance to each feature and its rank carried equal weight within the function, and so were multiplied by 0.5.

$$R = ((D_{et}/V_{dmax})*0.5 + 0.5*(Rank/V_{rmax}))^{x}*R_{max}$$

Landscape resistance values for distance to *Roads* were classified using four ranks (660, 3260, 15510, 41750 AADT for each road types (Department of Transport 2015); see Table 4.3.1 for rank order). Based on examination of previous literature (Berthinussen & Altringham 2012b), a maximum distance of 200m was set for the *Roads* layer. As resistance was expected to decline with increasing distance to *Roads* (the inverse of the expectation for *Linear Features*), we used the following function:

$$R = ((1- (D_{et}/V_{dmax}))*0.5 + 0.5*(Rank/V_{rmax}))^{x}*R_{max}$$

Where V_{rmax} is a constant which represents the highest number of ranks within the *Roads* layer, set to the highest AADT (41750; rank 4).

Once each resistance surface was created, we used Circuitscape (Version 4.0.5) to create current maps (McRae *et al.* 2008). To identify the functional connectivity for *R. ferrumequinum* at a local scale, we used a single roost location as the source layer. Since the exact movement patterns of the bats were unknown, e.g. the locations of potential foraging grounds, we generated a layer featuring concentric circles at 100m intervals from the roost to a maximum distance of 3km, using this as the target or ground layer. This enabled us to model movement scenarios from 100m to 3km, giving equal weight to each distance and direction.

4.3.3.3. Univariate and multivariate models

The optimal univariate models for each of the five environmental layers were determined, following the method detailed by Shirk *et al.* (2010). For each environmental layer, the value for both parameter functions, x and R_{max}, were increased or decreased (favouring the direction of increasing correlation) and revaluated after each iteration (100–161 variations per environmental layer with varying x (1–5) and R_{max} (1–10^{10}) values). This iterative process continued until an optimal model was found by examining and identifying the unimodal peak in the maximum Spearman's rank correlation coefficient between the parameter functions (x and R_{max}) output (Circuitscape current map) and the relative *R. ferrumequinum* activity data at Roost 1.

The resistance surfaces of the optimal univariate environmental layers were then combined into a multivariate model resistance surface for Roost 1. To incorporate the interactions between layers into this additive multivariate model, the parameter functions (x and R_{max}) of each layer were increased or decreased independently, while keeping all other layers constant, until a unimodal peak for each layer could be identified. This started with the univariate environmental layer with the highest correlation to *R. ferrumequinum* activity. If the parameter functions of a layer with a lower correlation value changed, then the iterative process started again, beginning with the univariate environmental layer with the highest correlation value, testing each iteration against the ground-truthed *R. ferrumequinum* activity data. The same parameter functions used in the univariate optimisation were used during the multivariate optimisation, and were increased or decreased until a unimodal peak was identified. This approach was taken because analysing every single parameter variation for each variable in relation to every other variable would have required an unfeasibly large number of model tests.

The univariate and multivariate processes were undertaken twice. First they used all nightly data collected during the acoustic surveys, illustrating general *R. ferrumequinum* movement and activity around their roost, over the entire night. Then secondly, they used data specifically

relating to *R. ferrumequinum* movement from their roost to their initial foraging ground at the beginning of the night, rather than movements during the entire night (activity recorded within the first hour after sunset), e.g. Pinaud *et al.* (2018). These two types of data sets were used to examine whether different environmental layers affected *R. ferrumequinum* activity in different ways, depending on the bats' behaviour.

4.3.2. Statistical evaluation and transferability

All statistical analysis were completed in R (version 3.3.0) (R Core Team 2016). Spearman's rank correlations were used to examine the relationship between relative bat activity recorded at each of the detector locations and the subsequent current density produced from the Circuitscape current maps for each model. Unlike Shirk *et al.* (2010), Spearman's rank correlations, rather than Mantel's correlations, were used because our response variable (bat activity) was not a matrix of distance based metrics (e.g. genetic distance). The univariate and multivariate models were initially built using 93 bat detector locations in the study area at Roost 1 (training roost). The successful transferability of a model can be defined as the ability for it to produce accurate predictions for areas outside that used for the initial training model (Justice, Covinsky & Berlin 1999). The transferability of the optimal multivariate model from Roost 1 was tested at Roost 2 – 4 by examining it against independent datasets collected within 3 km of each of these respective roost (between 33 and 38 bat detector locations). Using data that were not used to train or develop the models allows for a more stringent model testing, reducing the chances of overfitting, and makes the model a more reliable predictor of new data points (Xu & Liang 2001; Urban *et al.* 2009).

Like Pinaud *et al.* (2018), we wanted to investigate the accuracy of our connectivity models further by testing whether there would be a greater likelihood of *R. ferrumequinum* radio-tracking fixes occurring in more permeable areas of higher Circuitscape current, or whether they would be more randomly located in the landscape. Following the methodology outlined in Driezen *et al.* (2007), z-scores were created to examine whether the cumulative sum of the cost

of an individual reaching a certain location (i.e. each radio-tracking fix) was less than the mean cost of reaching all other points of equal distance from the roost (equidistant cost). For example, if the fix location was 1 km from the roost, we calculated the current value at this fix location and then compared it to the mean current value of all other locations at equal distance from the roost, i.e. all locations at 1 km from the roost. Thus, the analyses took into account the travel route and cost by each radio-tracked bat from the roost to each of their fix locations. To create the standardized z-score for each fix, we subtracted the mean equidistant cost from the Circuitscape current value at the fix location, and divided this value by the standard deviation of that mean cost. A positive value indicated that the fixes were on a route of higher functional connectivity (lower cost) than randomly selected locations. The results of the 191 fix locations were then compared with a normal distribution using a Shapiro–Wilk (*W*) test to examine whether they were significantly different from zero. As the radio-tracking data could have been accurate up to approximately 100m, we resampled the final model output to a 100m resolution and examined whether this influenced the result. The data were log transformed prior to analysis to achieve normality.

In addition, the optimal multivariate model output was compared against an expert opinion 'fly ways' dataset at Roost 1. This had previously been created, at the request of the Local Planning Authority and the Statutory Nature Conservation Organisation (Natural England 2010), by experts with local knowledge of bat activity in the region, who visually examined the landscape and selected areas of expected high functional connectivity for *R. ferrumequinum*. These 'fly ways' have been given additional protection from future developments and were designed for both local and larger scale movements. No radio-tracking data were used in the creation of the 'fly ways' presented in this study. To produce a comparison of the Circuitscape model and the expert opinion 'fly ways', we overlaid the optimal multivariate model output, and compared inside and outside the flyways that had high current (top 25%). The data were standardise by the distance of each detector to the roost. We then examined the relationship between the

optimum multivariate model, produced for *R. ferrumequinum*, with the median data from all other bat species recorded on each bat detector at Roost 1, to try to identify whether such a modelling approach and conservation efforts for a single key species would be beneficial for the entire bat community.

4.4. Results

Multivariate connectivity models provided a better description of the environmental layers around Roost 1 compared to any univariate model. The optimal univariate model's maximum per pixel resistance values differed from the multivariate model for three out of the five environmental layer types (Table 4.4.1). Similar results were obtained using early night, rather than all night, data only, except the maximum resistance values of *Land Cover* and *Linear Features* for the multivariate model were 10,000 and 50,000, respectively (Appendix 4.7.1).

Table 4.4.1: Per pixel resistance values for training roost location for both optimal univariate and multivariate models.

Environmental layer	Resistance values for the optimal univariate model	Resistance values for the optimal multivariate model
Land Cover	1000	10
Lightscape	1000	10^8
Distance to Rivers	1000	1000
Distance to Linear Features	10	25,000
Distance to Roads	10	10

The optimal multivariate model of general *R. ferrumequinum* movement could be transferred from one roost location to another, with all locations showing a significant correlation (Table 4.4.2). Nevertheless, there are variations between these locations.

Table 4.4.2: Spearman's rank correlation, the number of ground-truthed bat detector locations and model type for each of the four for *Rhinolophus ferrumequinum* (GHS) roost locations.

	Model type	No. of ground-truthed locations	Spearman rank correlation	p-value	Distance to Training Roost (km)	No. of GHS present in each roost during survey period
Roost 1	Training model	93	0.562	< 0.001	0	1187
Roost 2	Transferred model	38	0.448	< 0.01	13.5	816
Roost 3	Transferred model	36	0.336	0.03	76	435
Roost 4	Transferred model	33	0.360	0.03	31	260

The output Circuitscape current maps demonstrate the importance of linear features for the movement of *R. ferrumequinum* and highlight the impact of streetlights, while additionally identifying 'pinch points' within the landscape, allowing for spatial targeting of conservation measures in order to maximise conservation value (Figure 4.4.1). When comparing the raw data, we identify that *R. ferrumequinum* activity is 7.6% higher in the top 25% of predicted functional connectivity outside of the 'fly ways' compared with within them (Figure 4.4.2).

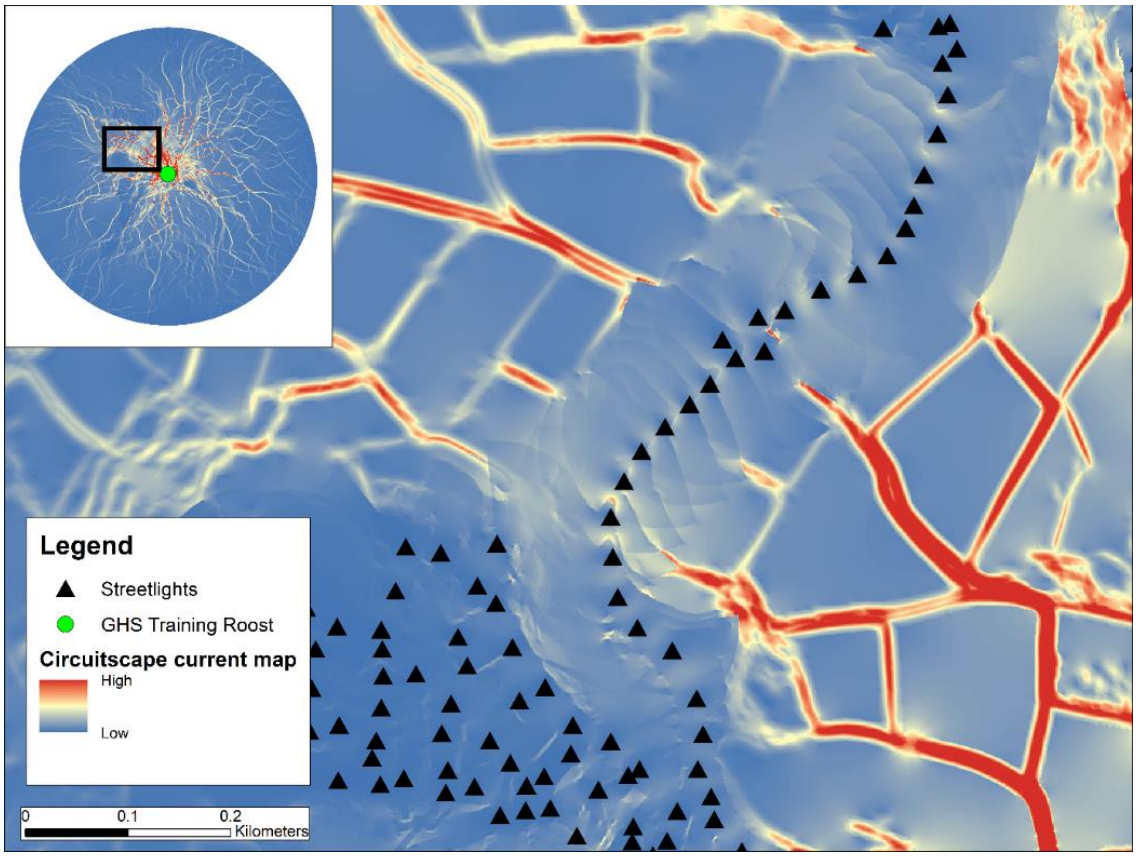


Figure 4.4.1: Image depicting functional connectivity for *Rhinolophus ferrumequinum* (GHS), pinch points, and the barrier effects of streetlights. Black triangles are streetlight locations, red indicates high, and blue indicates low functional connectivity. The inset map shows the locations of the GHS roost and area of street lighting being depicted (black square).

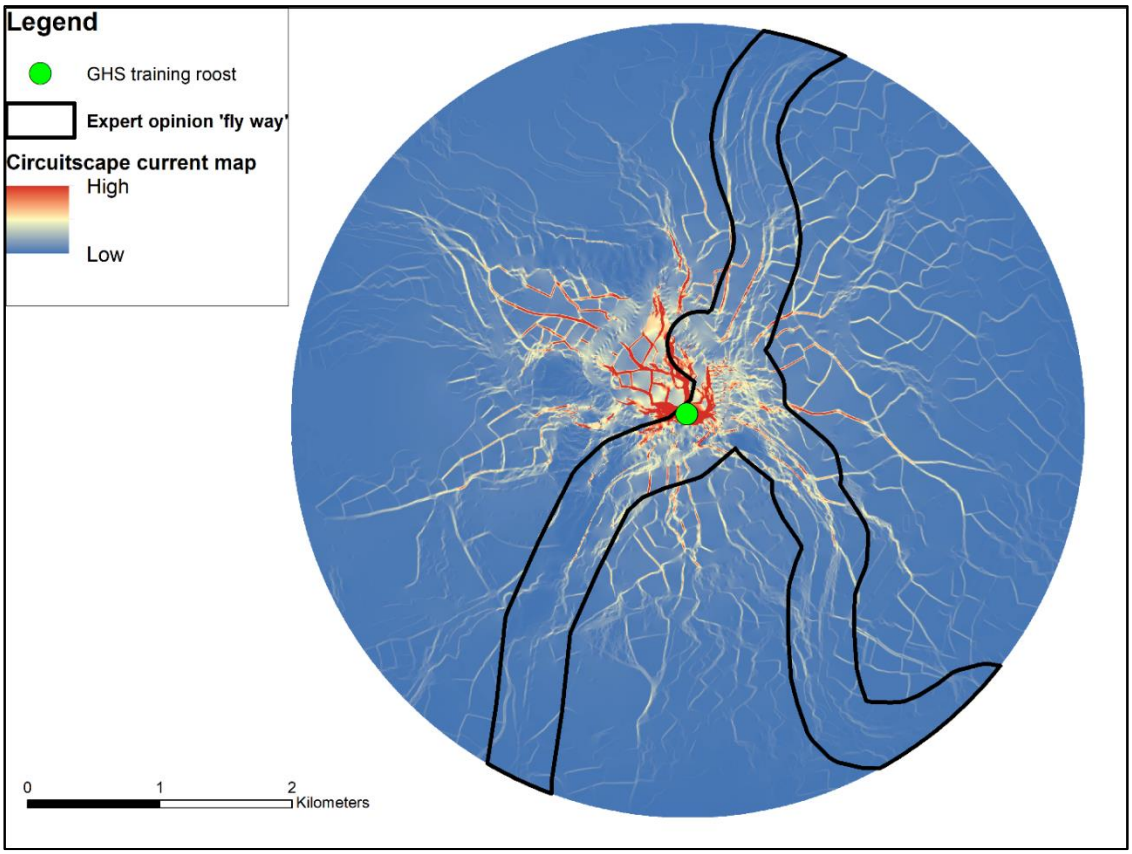


Figure 4.4.2**:** Circuitscape map centred on the *Rhinolophus ferrumequinum* (GHS) training roost location. Image illustrating the flow of current within the extent of the 3km map boundary, with the expert opinion 'fly way' layer overlaid on top.

During the radio-tracking studies, 191 *R. ferrumequinum* fixes were recorded within 3km of Roost 2 in Devon. The maximum fixes per individual was 31, with an average of 15. The results of ground-truthing the model using the 191 z-scores derived from the radio tracking data, showed a significant positive relationship with the Circuitscape current scores (mean z-score: 0.73, CI: 0.69–0.78, p-value: 0.016, *W*: 98). Similar results were obtained when the model output was resampled at a 100m resolution (mean z-score: 1.77, CI: 1.72–1.82, p-value: 0.003, *W*: 98).

Using *R. ferrumequinum* as an umbrella species and to explore the value of the modelling approach for the entire bat communities, we examined data for the other 10 species we recorded (*Barbastella barbastellus, Myotis spp., Eptesicus serotinus, Nyctalus noctula, Pipistrellus nathusii, Pipistrellus pipistrellus, Pipistrellus pygmaeus, Plecotus auritus,* and *Rhinolophus hipposideros*). The results of the multivariate model created (using all nightly data)

for Roost 1, identified that the median number of passes for all species recorded within the top quartile (i.e. 76-100%) of the observed Circuitscape current values (i.e. high current), were at least 125% higher than any of the lower three quartiles (Table 4.4.3).

Table 4.4.3: Median number of passes (interquartile range) of all bat species recorded per night per bat detector location at Roost 1, between 0 – 100% of observed Circuitscape current values.

Species	0% – 25%	26% – 50 %	51% – 75%	76% - 100%
Total species	37.12 (89.16)	53.57 (125.88)	25.29 (0)	120.50 (1111.39)
Barbastella barbastellus	0 (0.33)	0.38 (0.40)	0.80 (0)	0 (0.35)
Myotis species	1.43 (3.31)	2.93 (5.90)	2.80 (0)	1.53 (115.54)
Eptesicus serotinus	0 (0)	0 (0.06)	0.40 (0)	0 (0.35)
Nyctalus leisleri	0 (0)	0 (0)	0 (0)	0 (0.15)
Nyctalus noctula	1.80 (3.54)	4.75 (8.70)	6.20 (0)	0.80 (0.95)
Pipistrellus nathusii	0 (0)	0 (0.04)	0.20 (0)	0 (0.04)
Pipistrellus pipistrellus	7.50 (52.45)	28.0 (52.30)	11.0 (0)	21.70 (19.33)
Pipistrellus pygmaeus	5.0 (21.27)	11.08 (23.09)	6.80 (0)	8.26 (324.89)
Plecotus auritus	0 (0.33)	0.13 (0.63)	0.20 (0)	0 (0)
Rhinolophus ferrumequinum	2.38 (6.79)	8.92 (8.53)	6.0 (0)	47.40 (100.15)
Rhinolophus hipposideros	0 (0.33)	0.08 (0.52)	0.20 (0)	0.24 (1.41)

4.5. Discussion

Urbanisation and agricultural intensification are well documented to be causing a loss of connectivity within our natural environment (Millennium Ecosystem Assessment 2005a). In increasingly fragmented landscapes, it is vital that connecting routes, as well as habitat patches of high inherent value, are conserved. For our focal study species, the greater horseshoe bat, the results of our optimal multivariate model align with our current knowledge of its movement behaviour (Pinaud *et al.* 2018). We demonstrate that our *Linear Features* layer increase permeability within the landscape, whereas artificial night lighting decreases it (Duvergé 1996; Stone, Jones & Harris 2009; Day *et al.* 2015). However, the extent of the influence on functional connectivity in the landscape is unexpected. The final multivariate resistance value for the *Linear Features* layer indicates a dramatic decrease in the likelihood of relative *R. ferrumequinum* activity at increasing distances from the feature. The converse is true for the *Lightscape* layer, where streetlights were found to have a high impact on the permeability of the landscape for *R.*

ferrumequinum, which is in line with current literature relating to horseshoe ecology (Stone, Jones & Harris 2009; Stone, Jones & Harris 2012; Day *et al.* 2015). Similar results were obtained using only early night data, except that the maximum resistance of both *Land Cover* and *Linear Features* increased compared to the general movement of *R. ferrumequinum*. This highlights that, within the first hour after sunset, the activity of light sensitive bats, such as *R. ferrumequinum*, will be more tightly constrained to hedgerows and features that are more sheltered. At a local scale, these types of considerations could play a part of the success or failure of any future conservation action plans or mitigation measures.

The ready availability of large-scale data on, for example, weather and land cover means that macro-scale models, which often are based on very course resolution data about the target species, are commonly generated. Whilst valuable, for example in identifying migration corridors or highlighting areas likely to be most appropriate for a National Park (e.g. Roever, Van Aarde & Leggett 2013a), effective conservation also relies on fine-resolution data relevant to local planning decisions (Lechner *et al.* 2015), such as that provided here. Our models are relevant for other species of conservation concern, suggesting that the outputs can be of wider general use for conservation planning if appropriate umbrella species are selected. The numbers of records for non-target bat species were at least 125% higher at areas in the top quartile of the *R. ferrumequinum* Circuitscape current values compared to any of the lower three quartiles. We also demonstrate that spatially-targeted approaches to connectivity modelling can help to identify the locations of critical 'pinch points' within the landscape. For example, individual streetlight placements can have a major impact on the overall functional connectivity of the study areas, with the current passing through narrow corridors of suitable dispersal habitat (e.g. *Figure 4.4.1: Image depicting functional connectivity for* Rhinolophus ferrumequinum *(GHS), pinch points, and the barrier effects of streetlights. Black triangles are streetlight locations, red indicates high, and blue indicates low functional connectivity. The inset map shows the locations of the GHS roost and area of street lighting being depicted (black square).*Figure 4.4.1). However,

one limitation of the current study was that only streetlights, but not other lights e.g. vehicle headlights or security lights, were taken into account, owing to the lack of suitable spatial data. We highlight this as an area that is a priority for future research.

We evaluated the extent to which the modelling approach taken here represented an improvement over simpler approaches for identifying key corridors in the landscape. We found that although some of the important local areas for connectivity fell within the 'fly ways' based on expert opinion, many of them were missed. Yet these expert-opinion 'fly ways' have historically been given greater protection through the Local Authority's planning system than other regions. The model also identified some linear features as being important for functional connectivity that did not align with current ideas of optimal habitat (Duvergé 1996), e.g. intensively managed hedgerows surrounded by arable fields. However, while our results indicate that models can be successfully transferred from one area to another, with significant results, a precautionary approach should be taken. We therefore caution against transferring models from a 'training' area without any ground-truthing: where very different environmental conditions prevail, new models should be built (e.g. Roach *et al.* 2017). This will ensure that the resultant maps incorporate the interactions and non-linearity between predictor layers relevant to the specific locality.

This study illustrates that a relatively simple framework, and an iterative approach to connectivity modelling, permits the influence of landscape features to be visualised at a local scale. It therefore overcomes many of the difficulties encountered when trying to incorporate research into real-world decision-making by local planners (Opdam, Foppen & Vos 2002). Our approach has the potential to facilitate evidence-based policy and management. The resultant models can help planners and conservationists reduce human-wildlife conflicts, by applying mitigation measures strategically at locations likely to be most sensitive to species movement and future land-use change. Stakeholders can also use the modelling technique described here

as a predictive tool. For example, the relative impacts of alternative scenarios, such as the positioning of new housing or lighting schemes, the creation of woodland or the restoration of hedgerows, can be assessed through this modelling process, helping to achieve evidence-based wildlife conservation.

Environmental Impact Assessments are already meant to give consideration to the landscape context of a site, including the cumulative impacts of multiple developments. In practice, most work is conducted on a site-by-site basis. The approach outlined here provides a tool to incorporate functional connectivity into decision-making.

4.6. Acknowledgements

We would like to thank all of the citizen scientists and landowners who help with the research, and Anna David and Mike Symes who help obtaining land-owner permissions. Thanks are also due to the local authority ecologists, and to Sarah Jennings, Julien Sclater and Mike Oxford, who highlighted the challenges facing local planning authorities and provided feedback throughout the development of this modelling approach. We also thank the experts who helped to create the expert opinion model, the expert opinion fly-ways map, and to those who reviewed the paper. Fiona Mathews is supported by NERC Knowledge Exchange Fellowship (NE/S006486/1) and Domhnall Finch by a PhD studentship funded by the Vincent Wildlife Trust, the Devon Wildlife Trust, the University of Exeter, and the University of Sussex.

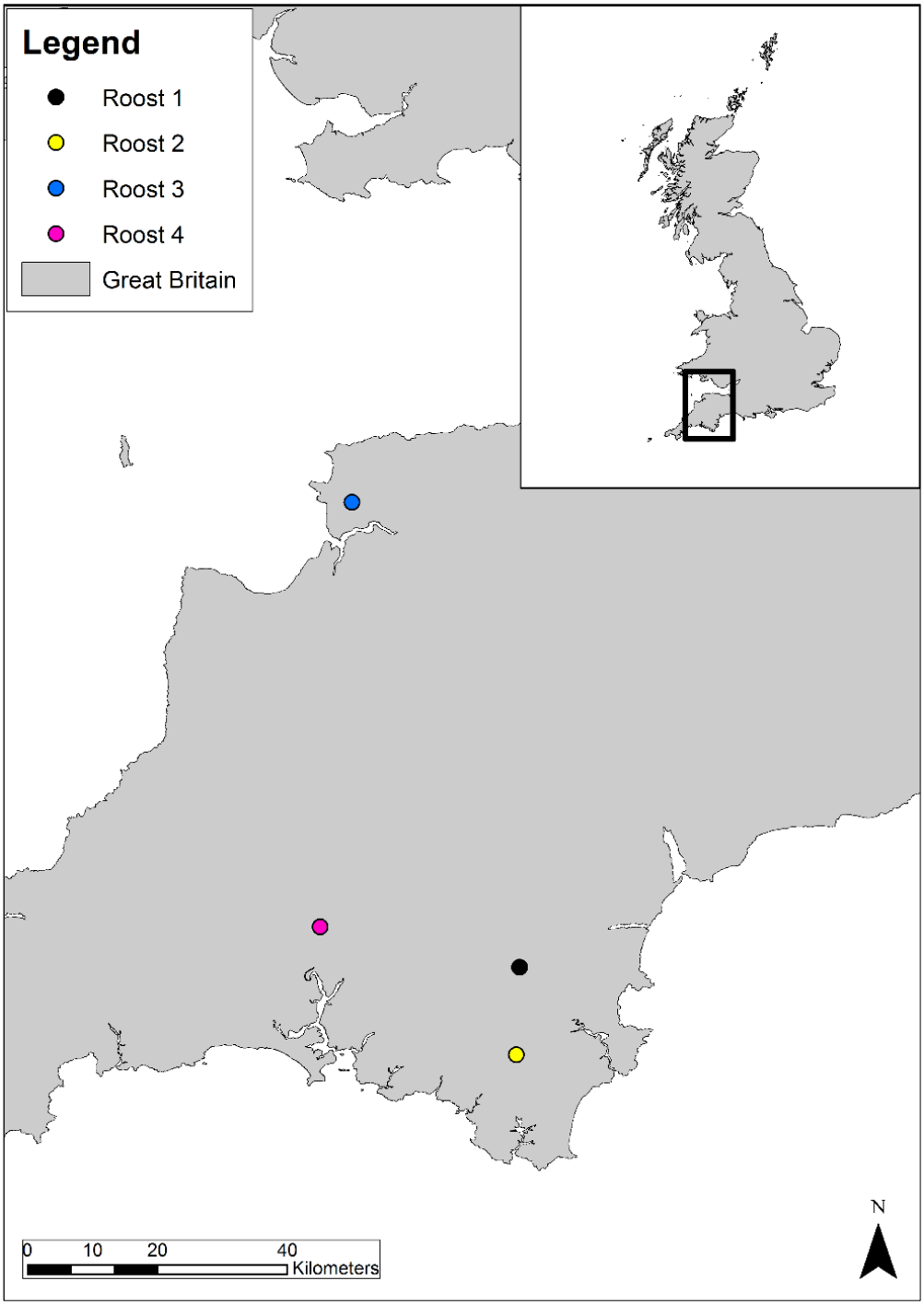
4.7. Appendices

Appendix 4.7.1**:** Per pixel resistance values for training roost location for both optimal univariate and multivariate models using commuting data only.

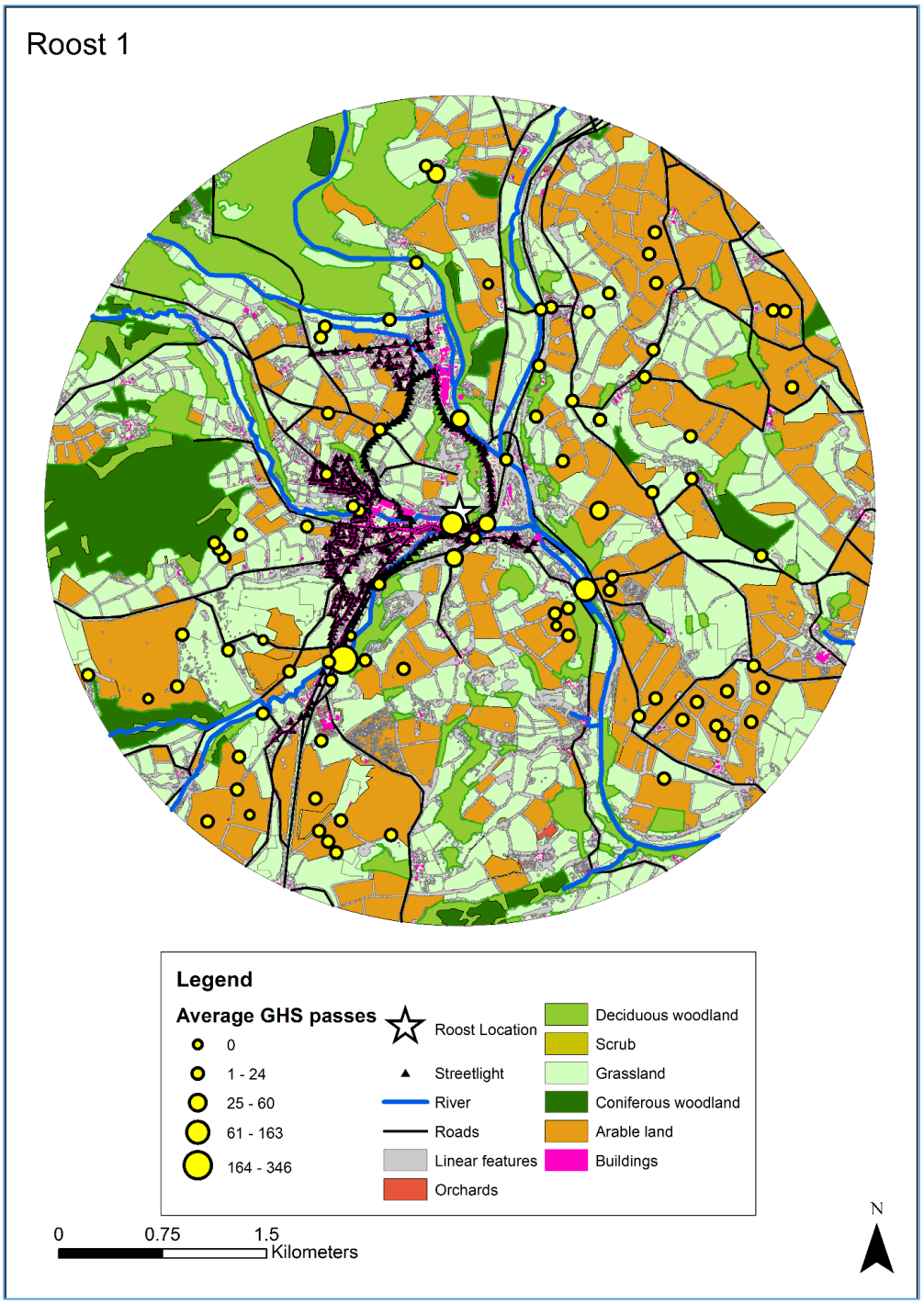
Environmental layer	Resistance values for the optimal univariate model	Resistance values for the optimal multivariate model
Land Cover	1000	10000
Lightscape	10^6	10^8
Distance to Rivers	100	1000
Distance to Linear Features	10	50,000
Distance to Roads	10	10

Appendix 4.7.2: Detector settings used for both the SMX-U1 and SMX-US microphones used in conjunction with SM2 and SM2 bat+ detectors (Wildlife Acoustics, USA) during the acoustic bat surveys.

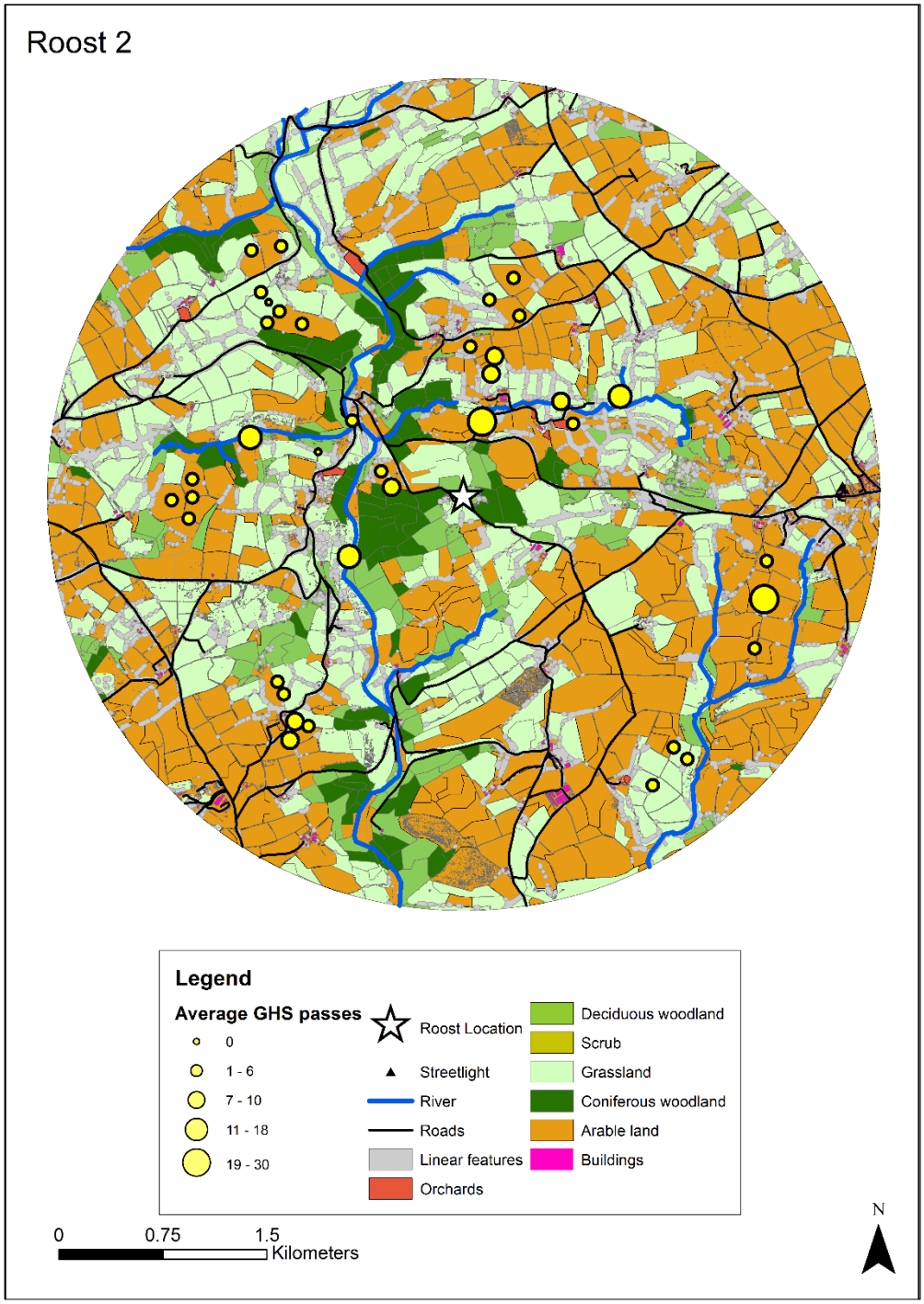
Detector settings	SMX-U1	SMX-US
Sampling rate	192000 kHz	192000 kHz
Gain	12dB	48dB
High pass filter	4 kHz	4 kHz
Low pass filter	Off	Off
Trigger level	18 SNR	18 SNR
Trigger window	2.0 sec	2.0 sec



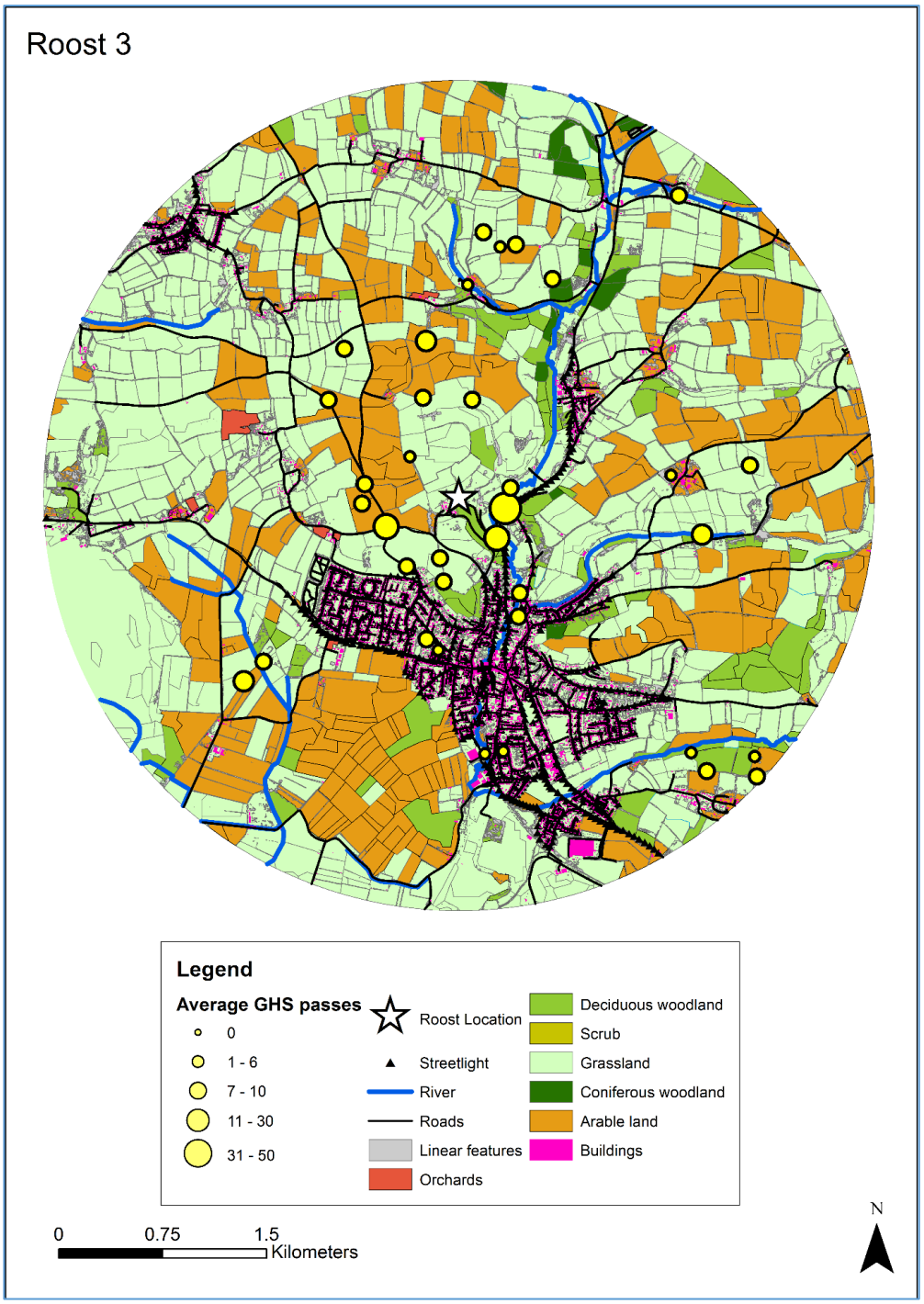
Appendix 4.7.3: Map showing the location of the four *Rhinolophus ferrumequinum* roost in Devon, England.



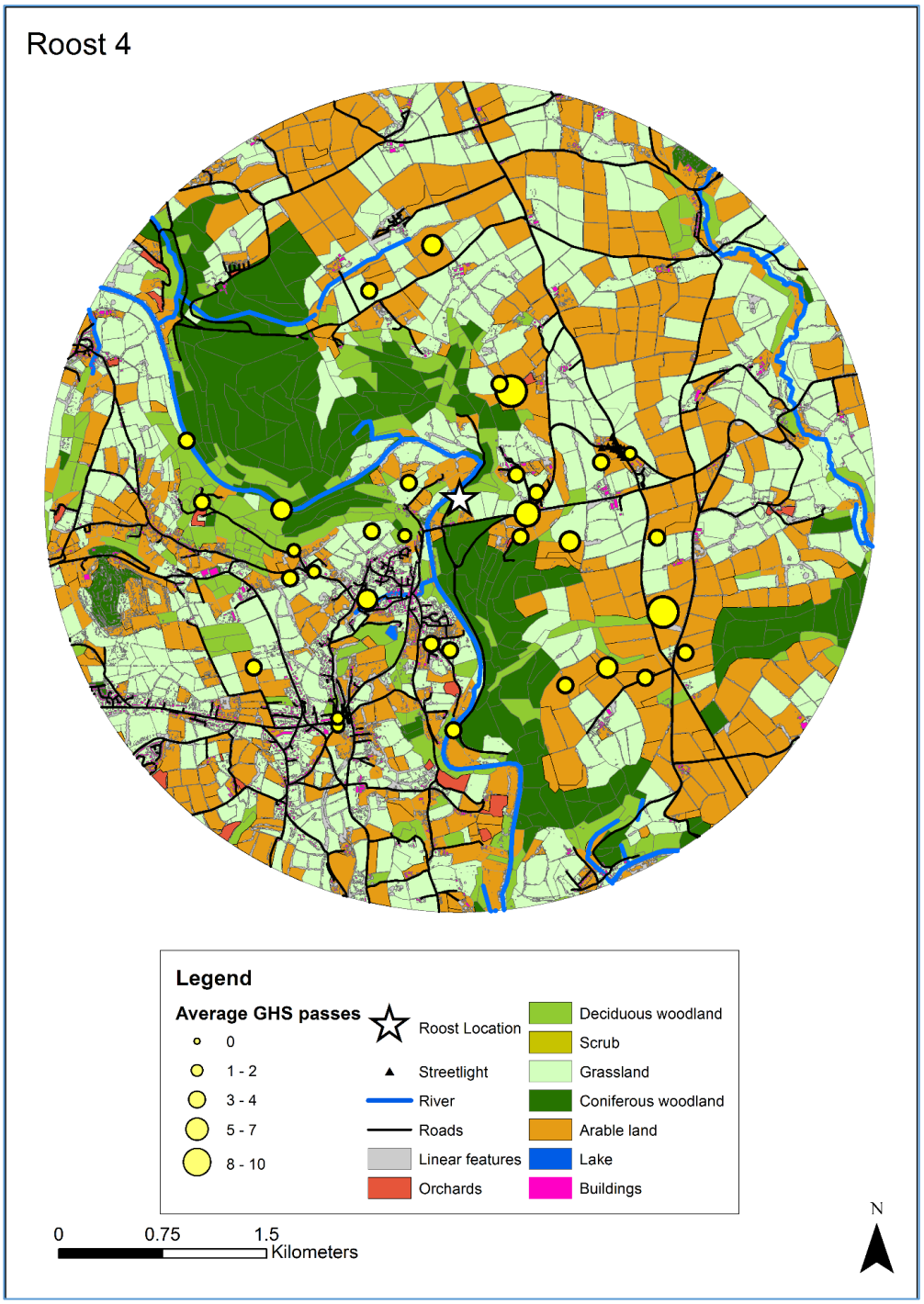
Appendix 4.7.4: Map illustrating the general mosaic of landscape features surrounding Roost 1 and the locations of the acoustic static bat detectors depicting the average nightly *Rhinolophus ferrumequinum* (GHS) activity. Map for illustrative purposes only.



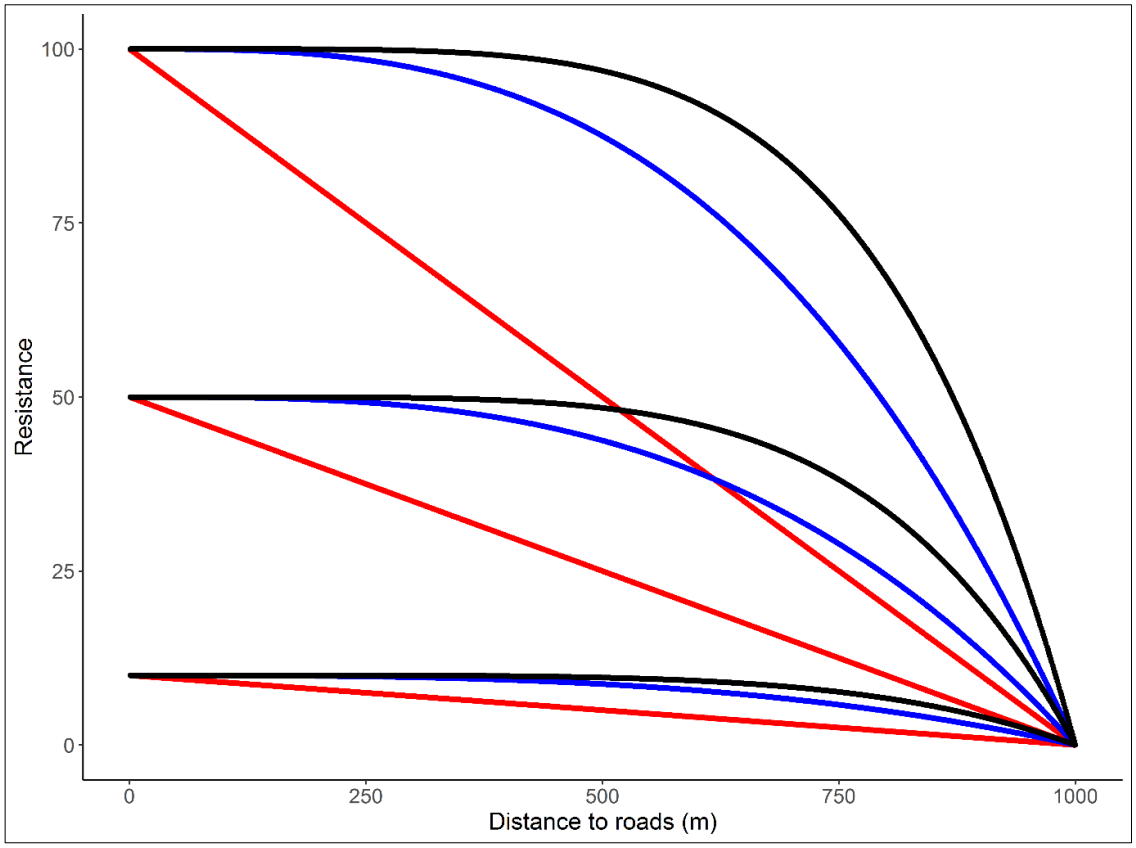
Appendix 4.7.5: Map illustrating the general mosaic of landscape features surrounding Roost 2 and the locations of the acoustic static bat detectors depicting the average nightly *Rhinolophus ferrumequinum* (GHS) activity. Map for illustrative purposes only.



Appendix 4.7.6: Map illustrating the general mosaic of landscape features surrounding Roost 3 and the locations of the acoustic static bat detectors depicting the average nightly *Rhinolophus ferrumequinum* (GHS) activity. Map for illustrative purposes only.



Appendix 4.7.7: Map illustrating the general mosaic of landscape features surrounding Roost 4 and the locations of the acoustic static bat detectors depicting the average nightly *Rhinolophus ferrumequinum* (GHS) activity. Map for illustrative purposes only.



Appendix 4.7.8: An illustration depicting how the relationship between different slope values (x) and the maximum distance to a GIS layer (V_{dmax}; in this case Roads), can influence the resistance value of landscape features. The correlation between these combinations of slope and resistance values can then be analysed against ground truthed data to identify which relationship best predicts how the species move through the landscape. As an example, this figure displays three separate maximum resistance scores (10, 50 and 100), with three slope values (1 (red), 3 (blue), 5 (black)). A slope value of one gives a linear response (constant rate of change irrespective to distance to the road), whereas a slope value of five gives an extreme concave response. Thus, illustrating greater resistance nearer the road, with a rapid decrease in resistance as you move further away from the road, with the rate of decrease controlled by the response curve of the slope value (x).

Chapter 5

Social network analysis and movement of bats during the hibernation season: a case study with greater horseshoe bats (*Rhinolophus ferrumequinum*).

5.1. Abstract

Social structures govern ecological networks and are fundamental to the interactions between individuals and groups, and consequently to the function of communities and ecosystems. These structures are often affected by the physical environment as the locations of these interactions can vary over time, particularly if there are high levels of fission-fusion in the population. Using a long-term dataset, we compared the social, temporal and spatial networks of hibernating greater horseshoe bats (*Rhinolophus ferrumequinum*). Over the course of 17 years, 2808 individuals were monitored, yielding a total of 5952 records at 21 roost locations. We aimed to investigate whether species attributes (sex, age and breeding status of females) affected the rate of associations between individuals, whether individual associations re-formed year on year, and to identify factors that influenced the movement of individuals between hibernation locations. Our results highlight that adult males were significantly more central in the network compared to any other attribute and are a link between individuals within the society. Consequently, they may play an important role in information transfer. While the network showed high modularity (community structure), yearly re-associations between individuals were not found to be significant. It does not appear therefore, that groups of animals move together between sites at a landscape scale, but rather that individuals move independently. Movements between hibernacula were associated with both the age and degree centrality of individual bats, with those more geographically isolated hibernacula playing a role for the movement of individuals at a landscape scale. Highlighting that despite low number of bats recorded in some of these smaller roost locations, they are of conservation concern because they increase the connectivity of the landscape.

5.2. Introduction

Social networks within populations are dynamic structures that are strongly influenced by the physical environment, as well as both the types of interactions which take place and the spatial distribution of the interacting individuals (Firth & Sheldon 2016; He, Maldonado-Chaparro & Farine 2019). For social species, the transfer of information between individuals (actively or passively), and the resultant creation and maintenance of beneficial relationships or competitive advantages for individuals, (Lewanzik, Sundaramurthy & Goerlitz 2019), is fundamental to a myriad of behaviours — ranging from mate selection, inter-species relationships, roost selection, identification of new foraging locations, and avoidance of predation (Wilkinson 1992; Doligez, Danchin & Clobert 2002; Aplin *et al.* 2012; Farine & Milburn 2013; Firth & Sheldon 2016; Ioannou *et al.* 2019). In turn, these can shape the outcome of entire population structures, making network analysis a useful tool for understanding, predicting and, potentially, manipulating population dynamics e.g. for disease management (Snijders *et al.* 2017; Gil *et al.* 2018). Identifying how these processes change temporally is crucial for obtaining a full understanding of network dynamics, however, most studies are of short duration, consisting of a single year or season (e.g. Vonhof, Whitehead & Fenton 2004). Examining the long-term trends within social networks could provide new insights into the variation which may occur between years or seasons (Kerth, Perony & Schweitzer 2011). Understanding these temporal relationships and social structures can allow for the identify areas of conservation importance leading to properly targeted conservation interventions. The outcomes of which can vary depending on the scale of the social networks are examined, with many studies only focussing on individual sites at a local scale rather than taking a landscape scale approach (e.g. August *et al.* 2014; Cox *et al.* 2016).

Bats make ideal focal species for social network analysis because they are widespread throughout the world (making up approximately one third of all mammal species), they are long-lived and most are social for at least part of the year. Previous social network studies have shown

that bats usually form fission-fusion societies, however there is high species-specific variation in social structures. For example, tree-dwelling species move frequently — in some cases daily — between roosting locations. The generation of communal knowledge about the locations of alternative roosting and foraging sites, through intra-specific transfer of information, is likely to be critical for species survival (Kerth & Reckardt 2003; Russo, Cistrone & Jones 2005; Carter *et al.* 2019). However, Kerth, Ebert and Schmidtke (2006) suggests that while group decisions about roost selection occurs by a majority decision, individuals can use their own knowledge and the behaviour of others to decide where to roost, allowing individuals to ignore majority decisions that are unfavourable to them. For instance, in populations of *Nyctalus lasiopterus*, Popa-Lisseanu *et al.* (2008) found that while the group composition changed with every movement between roosts, the social cohesion and structure of the overall population remained the same; However, August *et al.* (2014) showed that within the same woodland some species form separate social groups which rarely interacted with one another, illustrating the social divide which bats can create within continuous habitat.

The physical environment can play a significant role in roost selection and the social structures of bats. Rhodes *et al.* (2006) illustrated that the removal of a single tree that acts as a communal hub for the white-striped free-tailed bat (*Tadarida australis*) could cause a breakdown in connectivity within the wider landscape, as well as affecting group stability and viability. This highlights how using social structures can help predict how anthropogenic impacts could cause fragmentation and a reduction of encounter rates within populations (Snijders *et al.* 2017).

Greater horseshoe bats (*Rhinolophus ferrumequinum*) are a gregarious species, forming colonies during both the maternity and hibernation periods. In Western Europe, maternity colonies are typically found in buildings and classically contain mainly female bats and their offspring; males are found throughout the landscape in smaller roost locations. During the hibernation period, both sexes and all ages can be found together in cooler, underground sites (caves and mines)

(Hutson & Mickleburgh 2001; Dietz & Kiefer 2016). The literature suggests that there are three different types of hibernacula for *R. ferrumequinum*; these are differentiated by the age and sex of individuals, as well as the potential mating activity which occurs within them (Ransome 1968; Ransome & Hutson 2000). We know from ringing records that *R. ferrumequinum* often move between hibernation sites each year, and rarely travel more than 50km from maternity to hibernation roosts. Although, occasional movements of >60km are recorded (Ransome & Hutson 2000; Dietz & Kiefer 2016). To date, there has been no systematic study on hibernacula use by hibernating *R. ferrumequinum* that takes into account the unique identity of each individual.

Using a large geographic area and individual underground hibernacula as nodes, our study assesses, using social network analysis, how attributes (sex, age and female breeding status) of hibernating *R. ferrumequinum* are linked with the association rate between individuals. We hypothesised that the sex of individual bats would play an important role in the overall social structure of hibernating *R. ferrumequinum*. We also examine whether individuals re-associate with the same individuals on an annual basis, assessed the relationship between associativity and the spatial position of individuals between different hibernation locations, and predicted that the core hibernation sites would have the highest rate of movements recorded. In addition, identifying which attributes predict the movement of individuals between hibernation sites each year, and suggest how the social structures can help identify important areas for conservation.

5.3. Methods

5.3.1. Data collection

This long-term study took place between 2002 and 2019 at 21 hibernacula in south-west England (Figure 5.3.1). All the hibernacula fell within an area with a radius of approximately 30km. The monitoring of these hibernacula was undertaken by volunteers from the Wiltshire Bat Group. During each survey, unique identification metal rings were placed on bats (Natural England licence 2019-41471-SCI-SCI-3 and previous associated licences) that had not previously been

ringed; and the unique ring numbers were recorded for those bats that had been ringed previously. Only bats identified during these hibernation survey were used in the analysis. The age (subadult and adult), sex and breeding condition (females only) were also recorded during these surveys. Females were recorded as either parous or non-parous based on the size and presence of the nipples and false-nipples. Individuals were recorded as having breed if they were found to be parous in any year of the study. The age of the bats were determined based on fur colouration and density (adults have brown dorsal pelage with dense under-fur whilst juveniles and subadults have greyer sparser fur), the state of the wing membrane (shiny membrane that is slightly sticky and lacks extensive scarring in young animals), and the size of the metacarpal joints (which become progressively larger with age) (Dietz & Kiefer 2016).

A maximum of two visits (January and February) occurred at each location per year to minimise the risk of adverse effects from undue disturbance. Consequently, interdictions were not directly observed (e.g. Vonhof, Whitehead & Fenton 2004) but bats were considered to be associating if they occurred within the same hibernation location at the same time. These sorts of associations have been termed the 'gambit of the group' and are used when individuals are known to interact at a spatial scale of the group, with the interactions occurring at similar rates among individuals when in a group (Whitehead & Dufault 1999; Farine & Whitehead 2015). Individuals were excluded from the network analysis if they were only recorded once (e.g. Zeus, Reusch & Kerth 2018), or if they were recorded multiple times but were always solitary individuals. This allowed for more-accurate associations to be identified and excludes transient individuals that are only recorded in the network once.

5.3.2. Network analysis

All analyses were performed in R version 3.5.0 (R Core Team 2018). To describe the social structure, we created weighted, non-directional association networks using the "Simple Ratio Index" (SRI; Cairns & Schwager 1987) using the package "asnipe" (Farine 2013). The SRI index calculates the proportion of occurrences that two individuals (or nodes) are recorded together,

where the weighted associations (or edges) between individuals range from zero (never found roosting together) to one (always found roosting together). The index is calculated as X/(X+YAB+YA+YB), where X is the number of records during which bat A and bat B co-occurred in the same hibernaculum; YAB is the number of observation periods during which A and B were recorded at the same time but in different hibernacula. YA is the number of hibernacula that A occurred in without B over the time period both were known to be in the network; YB is the number of observations in which only B was observed. Using weighted SRI, compared to other association indices, has been shown to be better at describing true association patterns, making no assumption or biases with the data (Ginsberg & Young 1992), particularly when the associations are determined by individuals of the same group (Whitehead & Dufault 1999). This reduces the limitations that occur when using the 'gambit of the group' approach (Franks, Ruxton & James 2010). Owing to the length of this study, there were large temporal overlaps between individuals with observations of each individual potentially covering multiple ages which changed over the 17 years. To account for this we calculated a corrected version of the SRI for age by using a weighted average of each individual's age, where the weight is the number of times each individual was recorded as an adult and a subadult (e.g. Firth & Sheldon 2016).

To test whether associations between bats were linked to their attributes (sex, age and breeding status), we computed two descriptive metrics, degree and betweenness centrality. Degree is the simplest measure of centrality, calculating the number of connections between an individual (or node) and others in the network (Croft, James & Krause 2008). Individual bats with high degrees are more central in the network. Betweenness centrality counts the number of times an individual occurs on the shortest path between two other nodes in the network. High scores mean that the individuals are more likely to connect largely independent communities (Farine & Whitehead 2015) and that they are more vital for connecting different individuals within a network.

We created a single linear model for each response variable (degree and betweenness centrality) to test the relationship between node strength and our predictor variables (sex, age, breeding status, and the interaction between age and sex). Since the individuals in networks are unlikely to be independent of each other, the significance of associations was assessed by a comparison with random networks using permutation tests (Farine & Whitehead 2015). Each of the random networks were created using 100,000 permutations with a single random swap per permutation, while controlling for spatial and temporal factors (Whitehead & Dufault 1999). For every 100 swaps, a new randomised association matrix was extracted, resulting in 1,000 random networks. P-values were calculated as the proportion of times the coefficients of variations (CV) of the 1,000 random networks were greater than the CV of the observed data (Farine & Whitehead 2015; e.g. Zeus, Reusch & Kerth 2018).

The R package 'assornet' was used to test assortativity within the network for age, sex and breeding status (Farine 2014). This used weighted measures to examine whether individuals of the same kind, e.g. sex or age, were more often found together than expected by chance at a landscape scale. As with the linear models, the significance of these associations were tested by comparing the assortativity coefficients of the observed data with the randomly permuted networks.

5.3.3. Movement between hibernacula and community structure

Community structure was examined using the package 'igraph' to both visualise and detect ('infomap' algorithm) community modularity within the 21 hibernacula (Csardi & Nepusz 2006; Rosvall & Bergstrom 2008). Modularity can be derived from the proportion of edges (associations) that occur within a given community minus the expected proportion that would be observed were they randomly distributed throughout the network. The values obtained can range from zero to one, with values above 0.3 often being regarded as indicating social structure within a network (Whitehead 2009). However, computational simulations suggest a threshold of 0.5 (Shizuka & Farine 2016).

The movements of bats were examined within the network to identify the most important central hibernation locations and assess the bat characteristics that were linked with inter-site movements. To identify what influenced movement of individuals between hibernacula we used a binary GLMM model (link = logit), using the package 'lme4' (Bates *et al.* 2015). The response was the presence or absence of at least one movement within the study period. The predictor variables were age, sex, breeding status, degree/betweenness values, and the interaction between age and sex. AIC values and convergence parameters were used to determine the best-fit model. Individual *R. ferrumequinum* IDs were used as a random factor in the model to account for any non-independence of observations within site. We ran the above analysis twice, firstly using data from all 21 hibernacula and secondly using only data from eight core hibernacula. These sites had the largest number of bats within them and held 97% of the *R. ferrumequinum* records of this study (Figure 5.3.1).

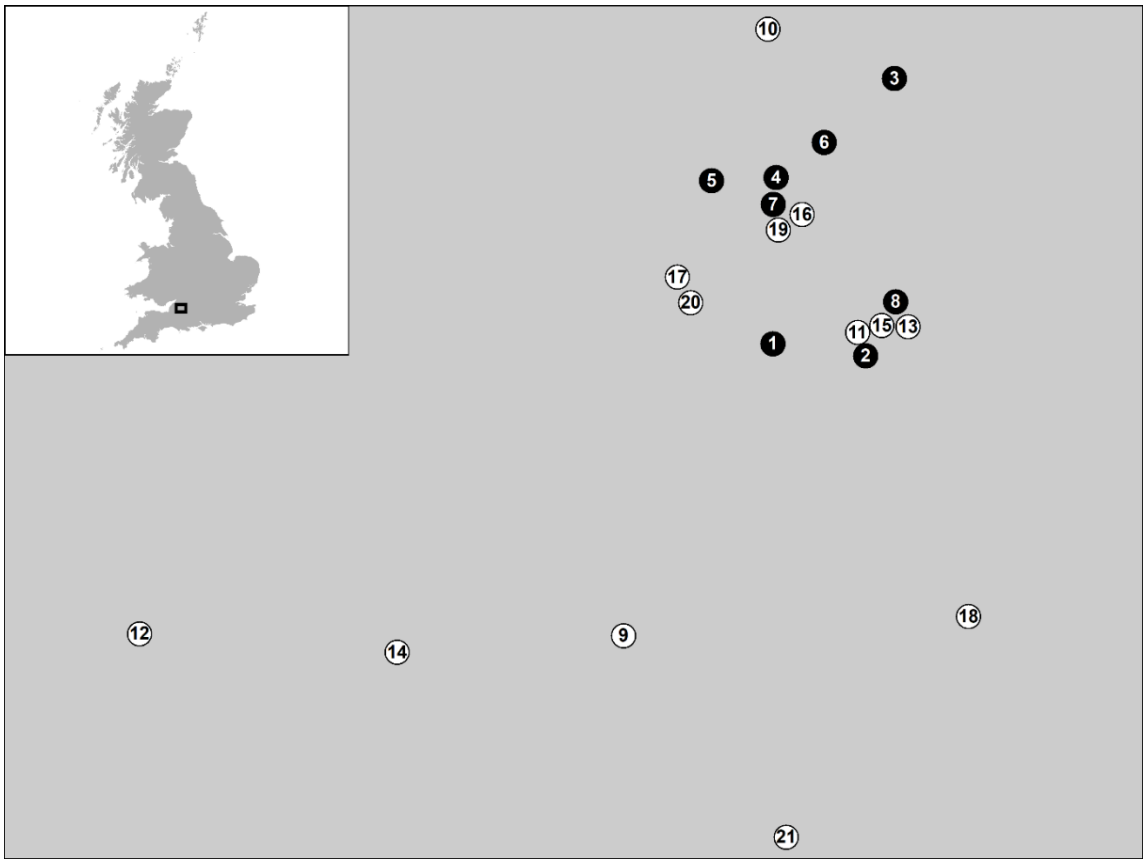


Figure 5.3.1: Map geographically representing the locations of all 21 hibernation roost surveyed. Core sites are shown in black, and non-core sites in white. Numbers in the centre of each circle are associated with the hibernation ID's in Table 5.4.1.

5.3.4. Temporal analysis

Lagged association rates (LAR) were calculated, using the package 'asnipe', to investigate the temporal persistence of any associations between pairs of individual bats, the step length was set to 365 days (Farine 2013). This gives the probability of any two bats being observed re-associating together within a set time period in the future (lags), while taking into account the number of times each individual bat was observed over the entire survey period (Whitehead 2008; Farine 2013). To allow further understanding of the LAR trends over the 17 years of this study, we calculated the lagged rate of association null by dividing the mean group size experienced by each individual by the mean number of total associates for each individual (the mean binary degree) (Farine 2013). This null value represents randomly associated bats: if the observed LAR drops below the null, the probability of bats re-associating between those lag periods is less likely than would be expected by chance. The standard errors for the LAR trends

were calculated by jack-knifing the data. Unlike the *Network Analysis*, the *Temporal Analysis* used all available bat data, including those excluded previously. This reduced the possibility of falsely biasing the LAR trends by only including data of frequently-observed *R. ferrumequinum* (Whitehead 2008).

5.4. Results

Over the 17 year monitoring period, 2,808 individual *R. ferrumequinum* were recorded in a total of 5,952 encounters, across the 21 hibernacula. The frequency with which these bats were caught ranged from 1 to 15 times (mean = 2.1; SD = 1.7; Appendix 5.7.1). The mean number of bats recorded each year was 351.4 (SD = 134.4), with a mean of 36.2 bats (SD = 25.9; range 0-221) being recorded in each hibernacula each year. The mean weighted degree was 36.2 per bat (SD = 25.9), the mean binary degree was 77.9 per bat (SD = 49.0), and the mean group size within the association matrix experienced by any one individual was 31.5 (SD = 18.6). Forty nine percent of individual bats recorded over the study period were female. While the majority of sites showed similar proportion of each sex some had comparably more males or females recorded (e.g. hibernacula number three, five and six), as well as five of the non-core hibernacula which were either solely male or female sites (Table 5.4.1).

5.4.1. Network analysis

Both linear models for degree and betweenness centrality were significantly different compared to the random networks (Degree: $coef_{observed}$: -3.47, mean $coef_{random}$: -2.88, *p*-value: < 0.001; Betweenness: $coef_{observed}$: 930.06, mean $coef_{random}$: -2.88, *p*-value: < 0.001). Degree centrality was significantly associated with the interaction between age and sex: adult males had higher association rates in the network compared with any other sex/age combination; no other variable was identified as having a significant interaction (*p*-value: > 0.05). When examining betweenness centrality, we found that breeding status (*p*-value: < 0.001) and the interaction between age and sex were significant in the linear model. For both degree and betweenness

centrality we found that adult males had higher association rates in the network compared with any other sex/age combination.

None of the predictor variables were significantly associated with assortativity (p-value: > 0.05 in each case), implying that there are not particular hibernacula that are predominantly segregated by a certain age or sex at the spatial scale of our study.

5.4.2. Movement between hibernacula and community structure

Overall, the number of mean bat movements between caves per individual was 0.6 (SD = 0.9) over the 17 years of this study. In total there were 940 records of *R. ferrumequinum* travelling between all hibernacula, 833 of which included movement to or from one of the eight core sites (Figure 5.4.1). Of those bats that did move, the mean number of movements recorded per individual was 1.5 (SD = 0.8); a maximum of seven movements between underground sites was recorded for one individual. Hibernacula number two had the highest amount of movement both to and from it, with almost 50% more records of individual bats moving to the hibernacula rather than from it (Table 5.4.1).

The mean degree centrality of *R. ferrumequinum* occurring in each of the 21 hibernacula ranged from 9.2 to 76.5, with seven out of eight core hibernacula being in the top eight uppermost ranking locations for higher centrality in *R. ferrumequinum*. The single other core hibernaculum, which is geographically quite central, was ranked 14th on the list (Table 5.4.1). Similarly, the core hibernacula showed on average higher betweenness centrality compared to non-core sites. However, the highest betweenness was observed at a non-core hibernaculum (hibernaculum number 11; Table 5.4.1).

When movements between all hibernation sites were examined both age (OR: 34.5, CI: 3 – 397, *p*-value = 0.005) and degree centrality (OR: 0.93, CI: 0.91 – 0.97, *p*-value < 0.001) were significant; highlighting that adult bats and those of lower centrality were more likely to use more than one hibernation site. However, when only using data from the eight core hibernacula

we do not see any significant relationship between the probability of movement and degree centrality (*p*-value > 0.05), though the relationship with age remains, with adults being considerably more likely to move (OR: 106.6, CI: 17 – 672, *p*-value < 0.001). These results are also highlighted in the raw data (Table 5.4.1), with the proportion of movements (compared to the total number of bats recorded in each hibernacula) higher in non-core hibernacula (max 67%) compared to core hibernacula, majority below 20%.

While there are high fission-fusion dynamics within network over the length of this study, modularity was also high (0.59) demonstrating a deviation from randomness. As illustrated in Appendix 5.7.2, there are several distinct, but overlapping, community structures. Appendix 5.7.3 and Appendix 5.7.4 also show the variations in *R. ferrumequinum* numbers recorded over the course of this study at each core and non-core hibernaculum.

Table 5.4.1: Table showing total number of *Rhinolophus ferrumequinum* (GHS) recorded, median roost size, mean degree centrality/betweenness values, proportion of bat movements, and proportion of each sex in each core and non-core hibernacula.

ID	Core or Non-core	Total No. of GHS recorded	Median hibernaculum size	Mean degree centrality	Mean betweenness centrality	Proportion of movement from:to hibernacula	Proportion of movements compared to total number of GHS (%)	Sex ratio – Female:Male
1	Core	1475	91	39	4006	49:51	16	49:51
2	Core	1407	72	34	3838	34:66	16	46:54
3	Core	965	112	76	2528	47:53	6	42:58
4	Core	763	53	32	3885	51:49	18	49:51
5	Core	385	23	17	2955	51:49	15	67:33
6	Core	361	23	28	2645	59:41	16	65:35
7	Core	309	30	28	2670	70:30	21	45:55
8	Core	113	7	27	3258	56:44	42	54:46
9	Non-core	62	23	28	2033	95:5	35	31:69
10	Non-core	47	4	21	1559	74:26	24	79:21
11	Non-core	11	2	20	6452	50:50	45	18:82
12	Non-core	11	1	18	1618	70:30	45	18:82
13	Non-core	11	1	17	1743	67:33	55	36:64
14	Non-core	11	6	9	523	75:25	18	9:91
15	Non-core	8	1	22	4114	50:50	50	63:37
16	Non-core	3	2	22	1185	50:50	67	100:0
17	Non-core	3	1	16	1150	25:75	67	33:67
18	Non-core	2	2	16	0	100:0	25	0:100
19	Non-core	2	2	9	74	100:0	50	0:100
20	Non-core	2	2	9	78	NA*	NA*	100:0
21	Non-core	1	1	24	1058	100:0	50	0:100

*No bats were recorded moving to or from this location.

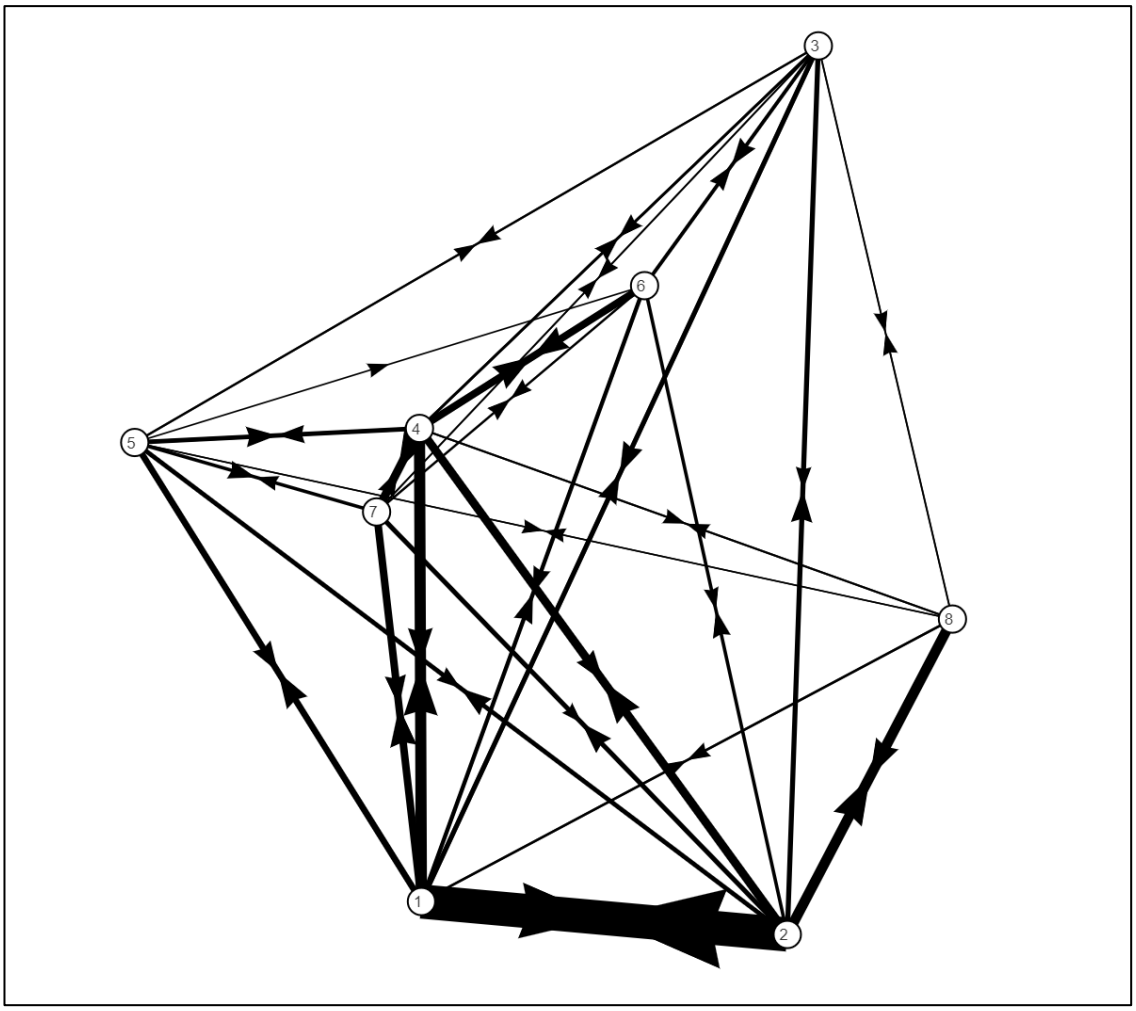


Figure 5.4.1: Movement of individual *Rhinolophus ferrumequinum* to and from core hibernacula. Thicker lines represent higher rates of movements between the hibernacula. Hibernacula are numbered according to the data in Table 5.4.1. Hibernacula are spatially distributed.

5.4.3. Temporal analysis

From our temporal analysis, approximately 22% of individual *R. ferrumequinum* re-associated with each other after the first year. This relationship rapidly decreased over time, and by year 17, < 1% of individual bat associations persisted (Figure 5.4.2). However, the LAR was consistently below the lagged rate of association null, indicating that the associations between individuals at the geographic scale of the study do not persist over time.

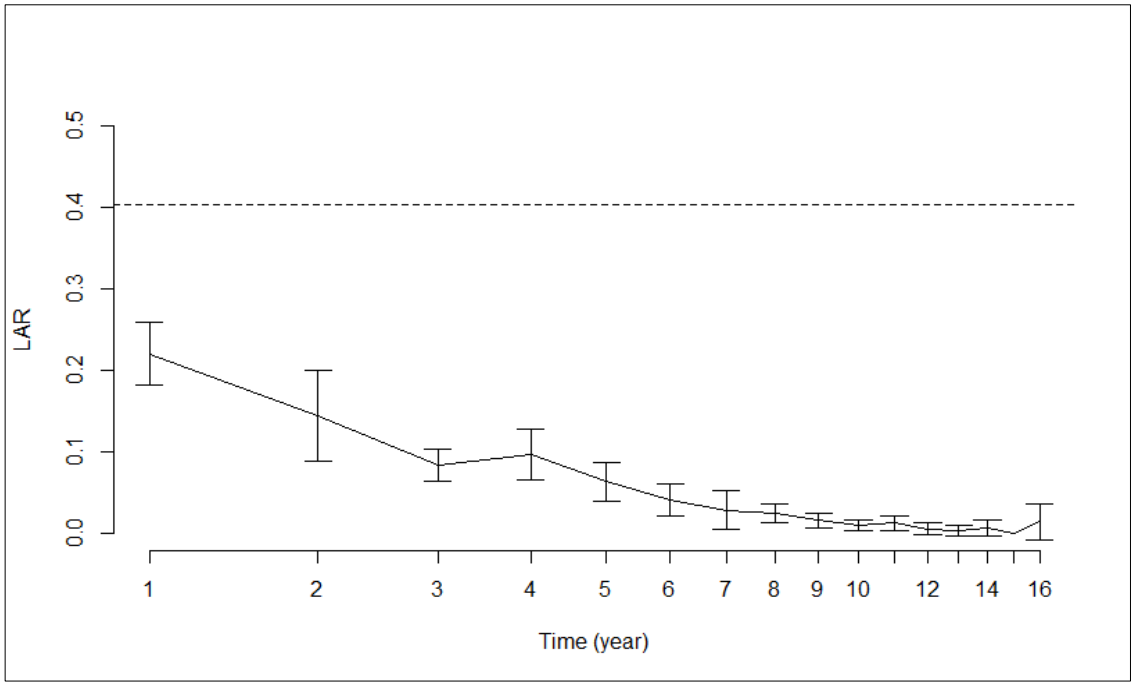


Figure 5.4.2: Lagged association rates (LAR) of hibernating *Rhinolophus ferrumequinum* over time. The solid line is the LAR with standard errors displayed and the dashed line is the lagged rate of association null.

5.5. Discussion

Overall, our results show that hibernating *R. ferrumequinum* are more associated with one another compared to chance, with males playing a key role in the social network within hibernacula; demonstrating the importance of long-term studies in identifying social structures and how an individual's attributes can influence their social interactions. Using the metric of degree centrality, adult males were significantly more central in the network, compared to any other attribute at the geographic scale of this study. This highlights the stark contrast in seasonal variation of *R. ferrumequinum* roosting ecology; where males are typically found on their own or in small groups of bats during the maternity season, with limited interactions with females (Ransome 1968; Ransome 1991), but they have been recorded in maternity colonies (particularly subadults). Conversely, during the hibernation season, our results show that adult males are more-likely to play a key role in social interactions, having multiple contacts with other individuals, whether this is for information-sharing, mating purposes or could potentially cause disease transmission. Strikingly, at a hibernaculum level, our results do not show that adult

females, particularly those that have bred before, form significantly close relationships with juvenile bats, as might be expected considering their close relationships during the maternity season (e.g. Rossiter *et al.* 2002). Similar to degree centrality, we show that betweenness is significantly associated with older bats, particularly adult males and those females that have bred before. We demonstrate that older *R. ferrumequinum* are more likely to be the link connecting individuals with otherwise low inter-connectivity, while juvenile bats of either sex are more likely to be independent. This could be due to the longevity of bat species, with the oldest *R. ferrumequinum* observed within these hibernation locations being at least 26 years old (ringed as an adult before the start of this study). These results support Kerth, Perony and Schweitzer (2011), who showed similar results for older Bechstein's bats (*Myotis bechsteinii*) when using a longer term data set.

These findings are illustrated by and concur with the data obtained on the movement of individual bats: adults were more likely to move between hibernation roosts compared to both juvenile bats and those with lower centrality scores (using all sites). Only when using data from the eight core hibernacula, could we show that degree centrality no longer becomes significant and only age shows a significant relationship with the movement of individuals. This highlights that it is those individuals of lower centrality values that are more likely to move between hibernacula outside of the larger core hibernacula. They have smaller, satellite roosts, which are usually geographically peripheral within a network of hibernation sites, as either the source or destination of most their movements. These results suggest that those bats in the larger core hibernacula have higher centrality because they interact with more bats within the system but do not usually need to travel outside of the core sites to maintain that centrality.

While the majority of the core sites had, on average, more central bats within them, several of the smaller satellite roosts had more bats with high centrality scores compared with one of the core sites. The highest betweenness centrality score was also recorded in a non-core

hibernacula, suggesting that these sites can act as locations to connect largely independent individuals as well as communities. These results stress the importance of these smaller sites to the movement and interaction of *R. ferrumequinum*, increasing connectivity and encounter rates at larger geographic scales.

Despite the high fission-fusion dynamics of *R. ferrumequinum*, seeing them disband each year to return to their summer roosting sites only to reform for the following winter, we identified several distinct, yet overlapping, community structures in the network. While we observed that some hibernacula showed some differences in the number of each sex recorded, we found that there were no preferences for same sex/age clustering, with communities made up of mixed sex social groups, similar to those found in the Spix's disk-winged bats (*Thyroptera tricolor*) (Vonhof, Whitehead & Fenton 2004). However, the data used in the present study does not examine individual clusters of bats found within hibernacula but rather focuses on larger geographic areas and tries to identify patterns at a landscape scale. Future research should concentration on social network analysis at an individual level to identify whether any patterns emerge of how hibernating *R. ferrumequinum* cluster together within individual hibernacula.

One limitation of this study was that our temporal analysis showed that the LAR was consistently below the lagged rate of association null, meaning that individual associations do not persist between years. One reason for this could be due to the ethical restrictions of conducting hibernation surveys, which could only occur twice a year; this is unlike other short-term studies that use automated techniques (e.g. Farine & Sheldon 2016; Firth & Sheldon 2016) or those occurring during the active breeding season (e.g. August *et al.* 2014; Zeus, Reusch & Kerth 2018), which are able to record more interactions. However, similarly to Popa-Lisseanu *et al.* (2008), these results could suggest that while group composition changes between years with every movement between hibernacula, the social cohesion and structure of the overall population remains the same.

There are many other factors when examining social networks, such as information and disease transmission, that can play a vital role in species survival. Bats are known to harbour various types of infectious diseases and viruses, harmful for both themselves as well as a host of other fauna including humans (Kruse, Kirkemo & Handeland 2004; Shi 2013). Our results illustrate that disease may spread quicker during the hibernation period when mixed sex groups are present in a roost, and due to the higher rate of connectivity, adult males are likely to have a higher probability of infection. Similar results were found for male Daubenton's bats (*Myotis daubentonii*) during the maternity season (August *et al.* 2014). Identifying how these patterns of annual social interactions can vary through time may have specific considerations when thinking about disease management, particularly when dealing with outbreaks that spread rapidly across populations and geographical areas, such as the white-nose syndrome in North America (Blehert 2012).

Nevertheless, there are considerable benefits to communal roosting and group decision-making, with the increased likelihood of information transfer. In bats, this has been shown to aid group foraging and to act as places where individuals can exchange information about resources in the wider landscape, as well as to help members coordinate roosting behaviour (Wilkinson 1992; Kerth & Reckardt 2003; Kerth, Ebert & Schmidtke 2006; Cvikel *et al.* 2015). To fully understand the social structure of *R. ferrumequinum* though, year-round patterns of association would be required to fully understand population dynamics. Further research would be required at hibernation roosts alongside those maternity colonies in close proximity to them. Firth and Sheldon (2016) showed that great tits (*Parus major*) that flock together during the winter, having higher social associations, are more-likely to breed in closer proximity to each other in the summer. They suggest that this could reduce energy expenditure on competitive interactions but also increase the possibility of mating between neighbours.

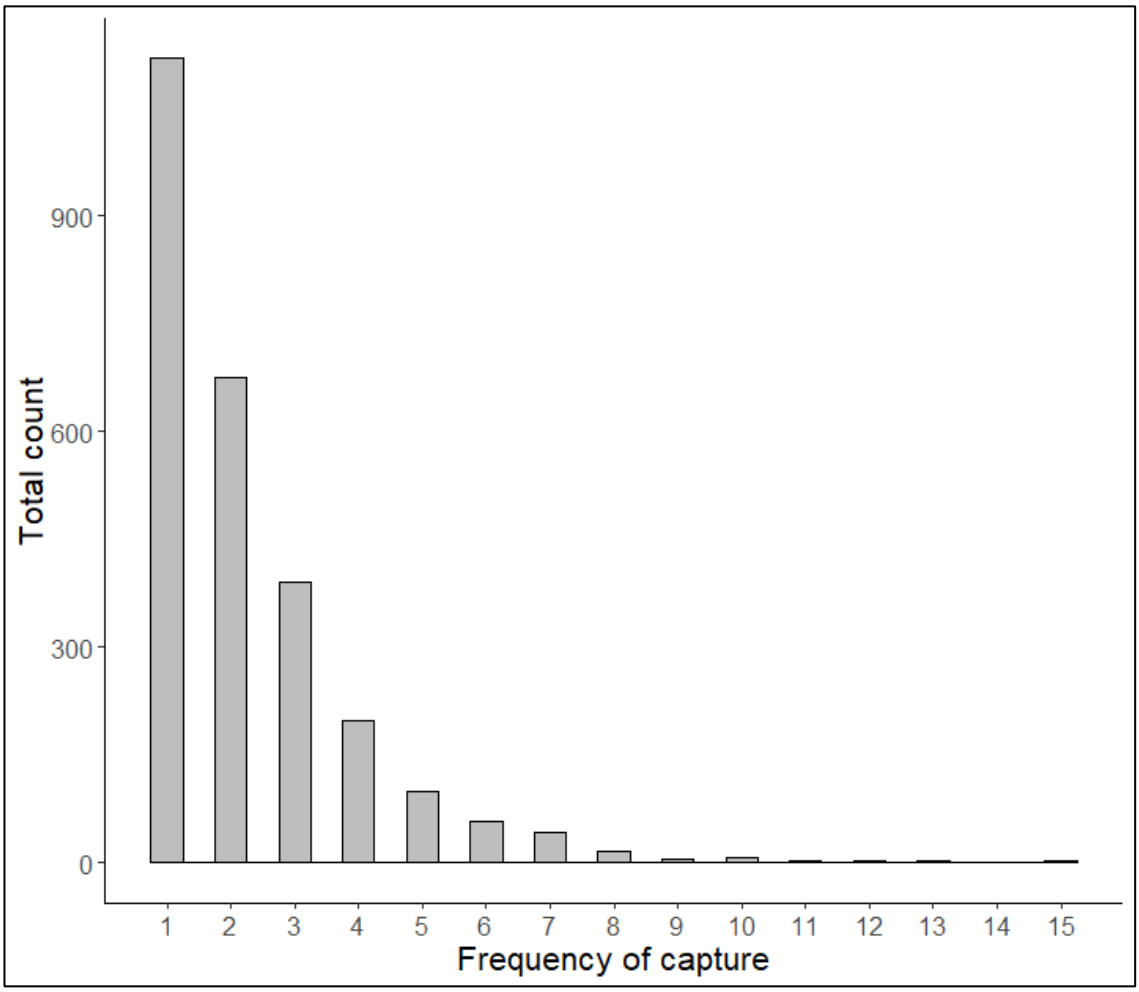
Understanding year-round patterns of species interactions and movement is essential for developing realistic management plans. This is particularly true for elusive species that travel between multiple locations within a single year (e.g. Hays *et al.* 2014), this includes cross-boundary jurisdictions, whether between countries, or between local authorities within a single country. The implementation of such management plans, and the collaboration of decision makers needs to be taken at a species level that considers their entire annual ecology, not just during the breeding period, as is increasingly recognised for long-range migrants (e.g. Bonter, Gauthreaux Jr & Donovan 2009; Chevallier *et al.* 2011; Tack *et al.* 2012). Understanding which physical features in the landscape act as barriers to these movements, or how alterations to the environment can influence social structures, can play a crucial role in their conservation (He, Maldonado-Chaparro & Farine 2019); for example, the red-backed fairy-wren (*Malurus melanocephalus*) whose habitats that are affected by wildfires were shown to have higher densities of network ties compared to those unaffected, due to habitat availability (Lantz & Karubian 2017).

For *R. ferrumequinum*, identifying movement patterns during the hibernation period is critical, particularly for smaller, satellite roosts that are quite often over-looked in the planning process due to the lower numbers of bats utilising them. We show that some of these sites, despite low numbers of bats being present, are key sites for social interactions and connectivity in the wider landscape at a population level. Loss of these sites could cause roost fragmentation and barriers in the landscape to species movement; it could also cause a decrease in the encounter rate of individuals due to a loss of resources and an increase in aggressive behaviour within a population (e.g. Macdonald *et al.* 2004). Additionally, as all of the main roost sites are interconnected, we highlight that, as a whole, the combination of these core and non-core hibernacula are vital for network stability and as such are conservation priorities.

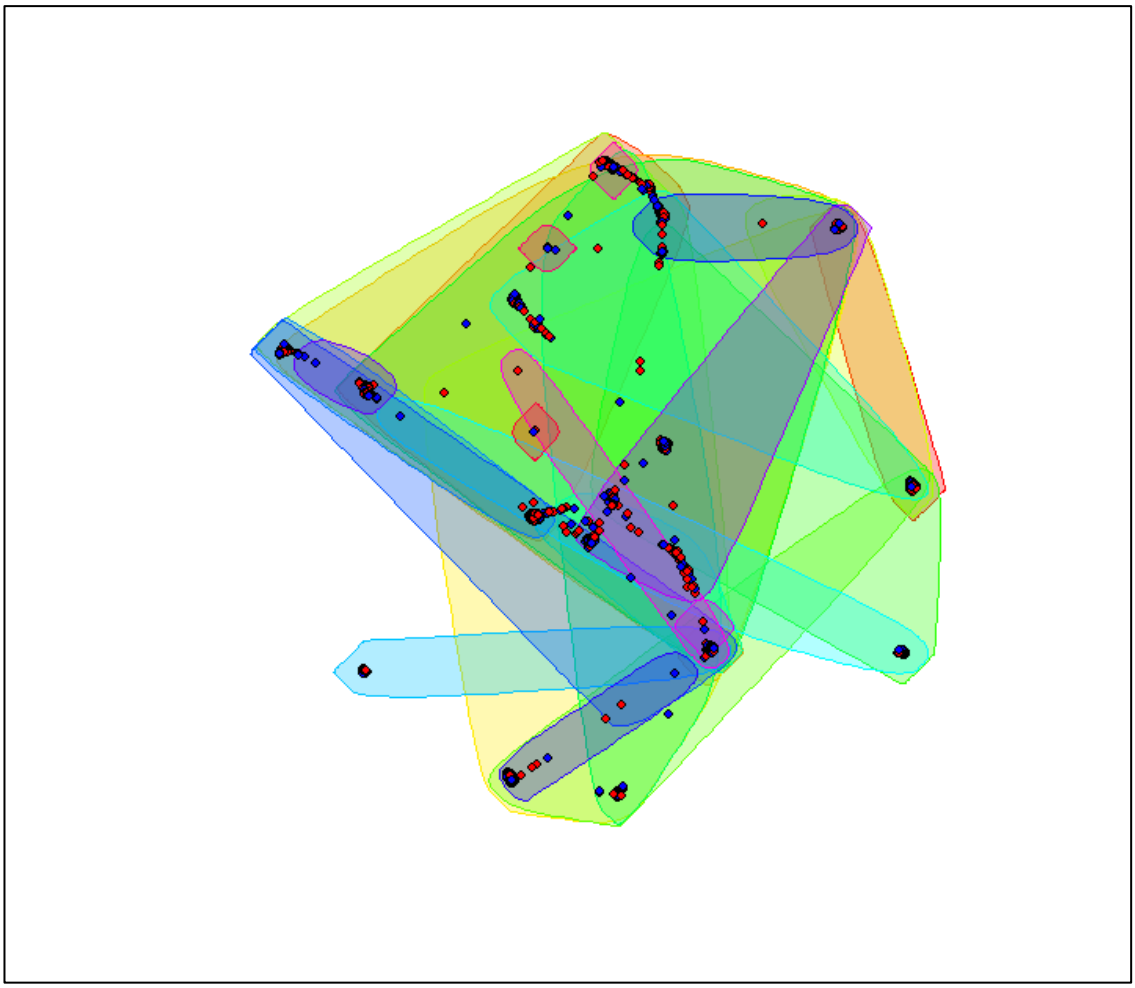
5.6. Acknowledgements

We are extremely grateful to all of the volunteers associated with the Wiltshire Bat Group who spent countless hours underground helping to collect this that. We thank all of the landowners who allowed us to access these hibernation locations. We thank Andrew Torsney for his comments on previous drafts of the paper. This research is supported by a PhD studentship funded by the Vincent Wildlife Trust, the Devon Wildlife Trust, the University of Exeter, and the University of Sussex.

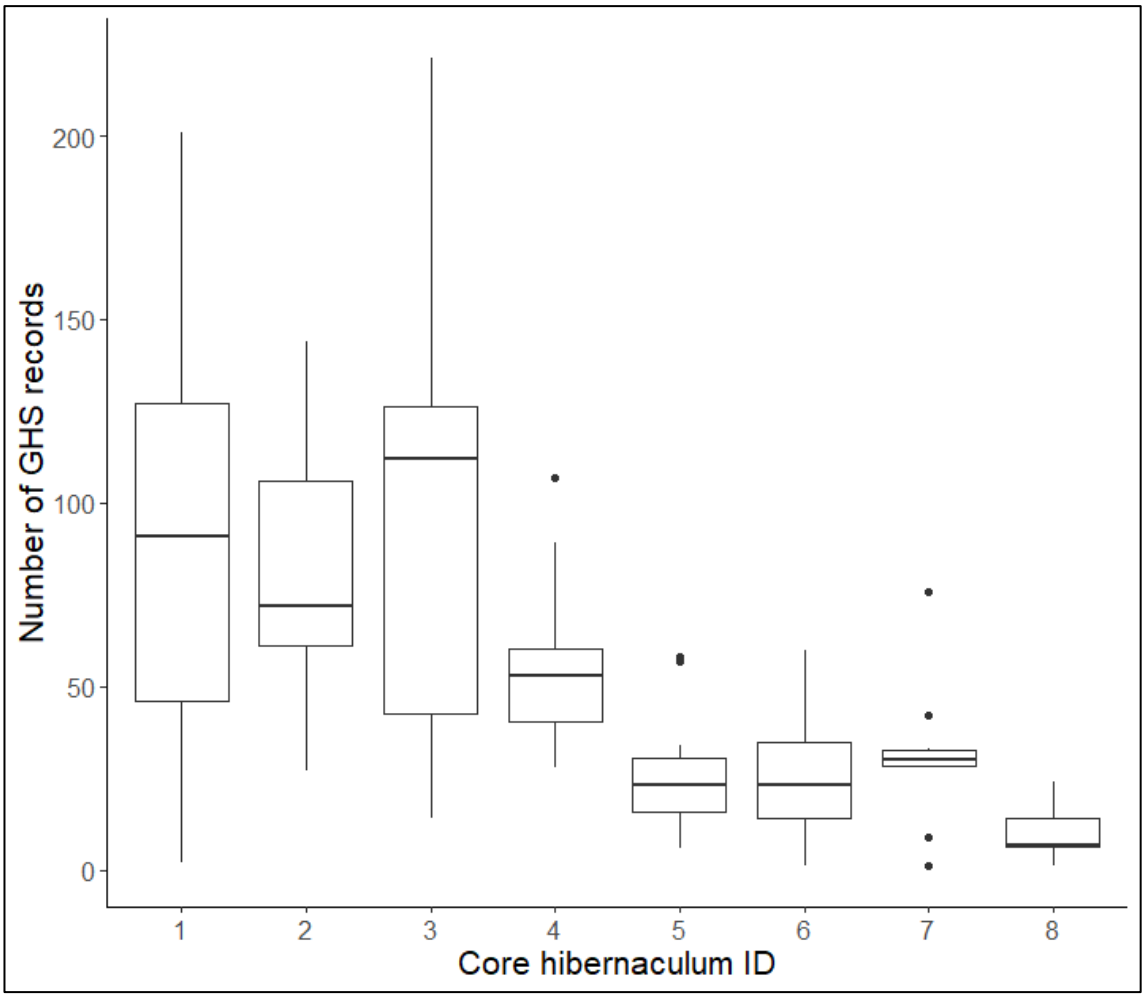
5.7. Appendices



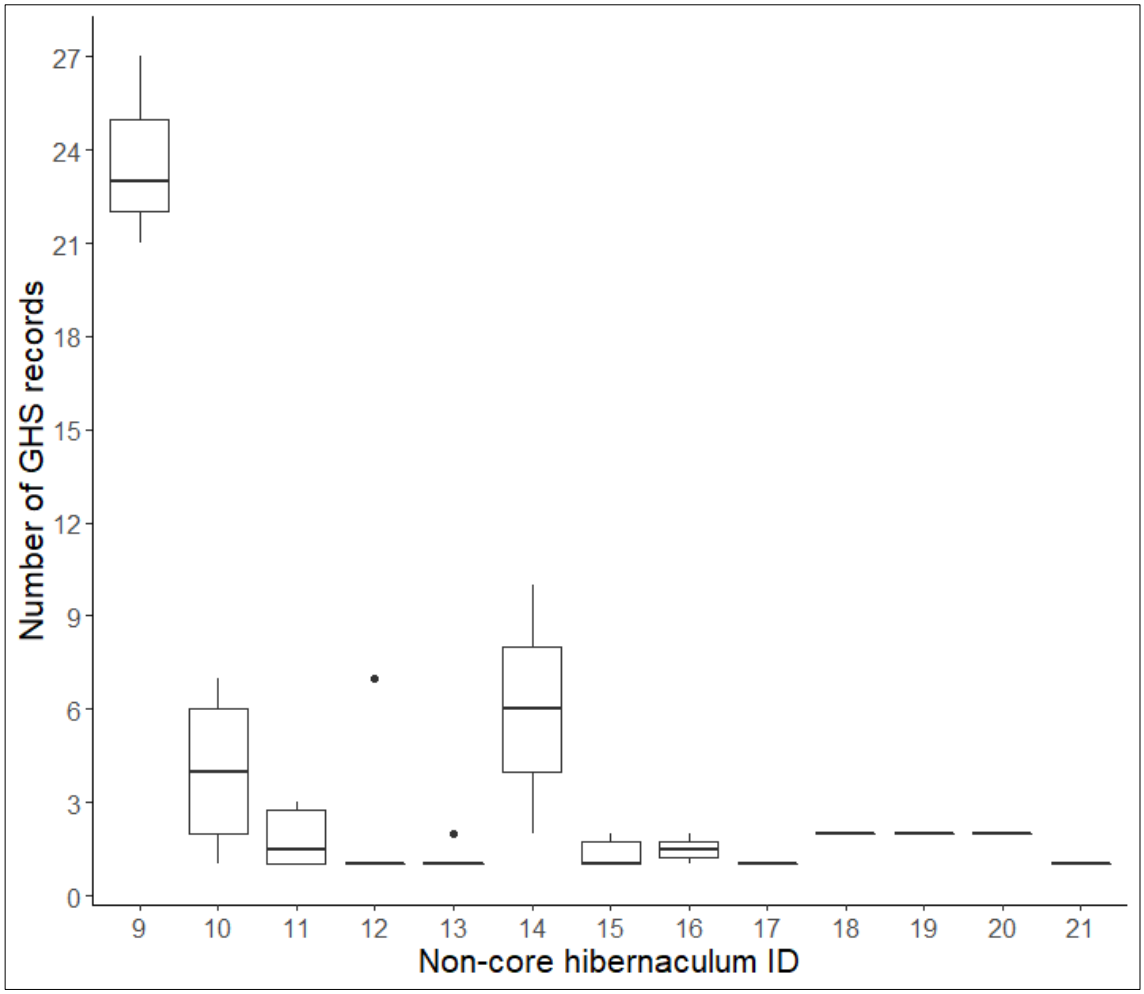
Appendix 5.7.1: Frequency of individual *Rhinolophus ferrumequinum* which were caught over the 17 years of this study.



Appendix 5.7.2: Depiction of the community structure observed within the social network of hibernating *Rhinolophus ferrumequinum*. Red dots are male and blue dots are female bats.



Appendix 5.7.3: Boxplots depicting median number of *Rhinolophus ferrumequinum* (GHS) caught at each core hibernaculum over the 17 years of this study.



Appendix 5.7.4: Boxplots depicting median number of *Rhinolophus ferrumequinum* (GHS) caught at each non-core hibernaculum over the 17 years of this study.

Chapter 6

Implications of endectocide residues on the survival of Aphodiine dung beetles — a meta-analysis.

An adapted version of this chapter has been published as:

Finch, D., Schofield, H., Floate K.D., Kubasiewicz L.M., and Mathews, F., 2020. Implications of Endectocide Residues on the Survival of Aphodiine Dung Beetles: A Meta-Analysis. Environmental Toxicology and Chemistry, **39**(4), 863-872.

6.1. Abstract

It is often difficult to compare studies examining the effects of endectocides on dung fauna because to different experimental approaches. For example, active ingredient (eprinomectin, doramectin, ivermectin, moxidectin) and formulations (injectable, pour-on, spiked). To gain a better understanding, we performed a quantitative meta-analysis using 22 studies to assess the overall effect of endectocide residues on the occurrence (presence/absence) and abundance of aphodiine dung beetles. Our results document a positive effect on the occurrence of adult beetles, indicating that adults tend to be attracted to dung with residues. Conversely, larvae are less likely to occur in the presence of residues. Thus, adults that colonize dung with residues either do not lay eggs or, more likely, the larvae that hatch from these eggs die early in development. Abundance of adult and larval stages were shown to be significantly reduced in dung containing residues. When individual endectocides were compared, only ivermectin demonstrated a significantly negative effect on the abundance of both adult and larvae, possibly owing to a small sample size for other agents. In laboratory studies, only dung 'spiked' with endectocides reduced the abundance of larva, whereas during field research, only pour-on applications were shown to reduce the abundance of larvae. The study further documents the non-target effects of endectocide residues on dung-dwelling organisms, provides robust evidence on the consequences of different application methods, and emphasises the need for standardised methodological techniques in future studies.

6.2. Introduction

Endectocides are among the world's most widely sold veterinary pharmaceuticals and have global application for the control of external and internal parasites affecting livestock. There is growing concern about resistance by target organisms to endectocides, and the consequent implications for farming (Kaplan & Vidyashankar 2012; Rose *et al.* 2015). Much less attention has focused on the potential environmental impacts of endectocides. Some endectocides can be poorly metabolised by the gut of livestock, with between 62 and 98% of the active ingredient being excreted as residue in dung (Canga *et al.* 2009). These residues can persist in the environment with a half-life of 240 days in laboratory conditions (Lumaret *et al.* 2012); under field conditions no degradation was detected up to 45 days post application (Sommer *et al.* 1992). This is concerning because residues can have significant impacts on both flora (Eichberg *et al.* 2016) and fauna (Iglesias *et al.* 2006; Jensen, Diao & Scott-fordsmand 2007) in the natural environment.

Under Phase II Environmental Risk Assessment guidelines (European Union 2009), the risk of veterinary pharmaceuticals to non-target species of dung-breeding organisms are assessed in single-species laboratory studies (Tier A testing) (VICH 2004). If a specific exposure threshold is exceeded in Tier A testing, additional testing is mandatory, using multispecies communities of dung-breeding organisms under more realistic field or field-like conditions (Tier B testing) (Floate *et al.* 2016). Specific risk threshold to the dung fauna can include mortality, reduced fecundity, impaired behaviour, and delayed development.

Other than this broad requirement, there is no standard methodology for Tier B tests (Jochmann *et al.* 2011). Researchers may use dung pats that differ in size and number from different species of animals fed on different diets. Studies may be performed at different times of the year with taxa identified to different levels of taxonomic resolution (e.g., family vs. genus vs. species). In addition, endectocides include both avermectins (e.g., doramectin, eprinomectin, ivermectin) and milbemycins (e.g., moxidectin) that can be formulated and administrated to livestock as oral

pastes, injections, extended-release injections, pour-ons, and sustained-release boluses (Herd 1995; Forbes 2013). All of these factors influence faecal concentrations of endectocide residues entering the environment (Lumaret *et al.* 2012) and the interpretation of results (Jochmann *et al.* 2011). Relatively few studies have directly compared the non-target effects of different endectocides (Hempel *et al.* 2006; Webb *et al.* 2010) or of the same endectocide in different formulations (Herd, Sams & Ashcraft 1996).

Depending on their concentration, faecal residues may be lethal to the organisms that colonize the dung, and their offspring that develop within the dung. They may also affect behaviour, fecundity, and developmental times. Residues also have been variously reported to attract or repel insects from contaminated dung (Holter, Sommer & Grønvold 1993; Floate 2007; Rodríguez-Vivas *et al.* 2019). Generally, however, faecal residues are reported to reduce the richness and abundance of diverse insects (especially species of Coleoptera, Diptera and Hymenoptera) and other organisms in dung (Floate *et al.* 2005; Lumaret *et al.* 2012; Nieman *et al.* 2018). Nevertheless, there can be considerable variation between studies in terms of the size and direction of the effects (Halley *et al.* 2005; Webb *et al.* 2010; Scheffczyk *et al.* 2016; Rodríguez-Vivas *et al.* 2019).

The effect of residues on dung beetles (Coleoptera: Scarabaeidae: Scarabaeinae, Aphodiinae) is of particular interest. They are typically among the most prominent insects present in fresh dung in terms of both abundance and biomass. Their feeding and breeding activities accelerate the removal of dung from pastures (Anderson, Merritt & Loomis 1984; Wall & Strong 1987), thereby increasing grazing area and encouraging the growth of healthy grass through soil aeration and nutrient recycling. Consequently, they help to increase the carrying capacity of pastures and reduce the risk of disease transmission (Bornemissza & Williams 1970; Herd 1995; Nichols *et al.* 2008; Beynon *et al.* 2012). Dung beetles are also key prey items for a number of species including the greater horseshoe bat (*Rhinolophus ferrumequinum*; accounting for approximately 33% of

the bats diet; Jones 1990; Flanders & Jones 2009) and choughs (*Pyrrhocorax pyrrhocorax*) (Roberts 1982; Meyer 1990; Young 2015). The presence of dung beetles has been shown to reduce numbers of pest flies breeding in dung by 58% (Beynon, Wainwright & Christie 2015) and to reduce the prevalence of cattle nematode infections by 55-89% (Fincher 1975). The resulting economic benefits have been estimated to equate to £367m a year in the UK alone (Beynon, Wainwright & Christie 2015). The impacts of endectocide residues are therefore important to the global agricultural economy.

Studies that report on the effects of faecal residues on dung beetles often include data for species in the subfamily Aphodiinae. This is likely because they are common in livestock dung across North America, Europe, Asia and northern Africa; with most aphodiines easy to identify to species level. Additionally, *Aphodius constans* Duftschmid has been approved by the Organisation for Economic Co-operation and Development (OECD) as a model test organism to assess the toxicity of faecal residues on dung-breeding organisms (Hempel *et al.* 2006; OECD 2010). The collective body of literature on the non-target effects of faecal residues to aphodiines includes diverse — and occasionally contradictory — results, possibly owing to differences in experimental design (e.g. Floate, Colwell & Fox 2002; Webb *et al.* 2010).

In the present study, we conducted a meta-analysis to understand better the overall responses of aphodiine beetles to endectocide residues in livestock dung. This subfamily were specifically chosen as a model group for our meta-analysis because of the considerable amount of raw data already available in the literature and they are one of the few dung-breeding insects for which an OECD guidance document has been produced. The analyses were specifically designed to assess the effect of different endectocides on the occurrence (presence/absence) and abundance of individuals, with consideration given to both to larval and to adult life stages. The analysis of beetle abundance incorporated and examined data from multiple studies

representing both field and laboratory experiments, which used different formulations (pour-on, injectable (in cattle), mixed in (i.e. 'spiked' dung)).

6.3. Methods

6.3.1. Data source and selection

Literature published in any language between 1990 and 2016 that reported the impact of endectocides on the abundance and occurrence (presence or absence) of aphodiine species was identified using the databases ISI Web of Knowledge and Google Scholar. MeSH (Medical Subject Headings) search terms were as follows:

('aphodiine' OR species taxonomic names) AND

('endectocide*' OR 'anthelminthic*' OR specific name of an endectocide) AND

(cattle OR cow OR sheep OR livestock).

These terms are an example of what was searched; exact search terms are given in the Appendix 6.7.1. The abstracts of each paper were reviewed to identify studies that: (1) reported data for aphodiine beetles, (2) examined endectocides, and (3) incorporated use of control (uncontaminated) dung.

The literature search identified 149 papers of which 27 matched the above three criteria. The papers that they cited, plus the papers that cited them, were cross-checked to identify 10 further papers suitable for inclusion. Requests for their raw experimental data were then sent to the authors of these 37 papers. Responses received for 11 papers provided > 25,000 individual rows of raw data. Additional responses identified papers for which data were no longer available, or which were not available in a suitable format.

To qualify for inclusion in the analysis, studies had to present key summary data (mean abundance per treatment type, standard deviation (SD), number of samples, and/or p-value);

provide information that permitted the calculation of these values; or provide raw data. For statistical rigour, we limited our focus to compounds represented in at least two datasets. At the end of this screening process, the data used in our meta-analysis comprised 31 individual data sets from 22 studies spanning 13 countries (Table 6.3.1).

Each data set assessed the effect of endectocide products on aphodiine beetles in cattle dung, but were otherwise diverse in nature (Table 6.3.1). Most data sets examined the effects of ivermectin (n = 22) with much less data being available for moxidectin (4), doramectin (3) and eprinomectin (2). In some cases, endectocides were added directly to the dung ('spiked' dung) rather than using dung collected from treated animals. Some studies placed known numbers of beetles into dung to assess the insecticidal toxicity of residues under lab conditions, whereas other studies used pitfall traps in the field to test the attraction or repulsion of beetles to residues. A further group exposed dung in the field to egg-laying adult beetles and then recorded subsequent numbers of larval or adult beetles recovered from the dung. There was also variation in the post-application period during which dung was collected from treated animals; the species of aphodiine beetles examined; time of year; and environmental conditions (humidity, soil pH, temperature). Additional information on variation of cattle breeds, diet and endectocide dosage used in each study can be found in Appendix 6.7.2. Thus, an aim of the meta-analysis was to detect general patterns of endectocidal effects on aphodiine beetles that might be otherwise masked by variation across individual studies, through the use of co-variate analysis.

Table 6.3.1: Description of the 22 studies included in the meta-analysis.

Source of data	**Endectocide**	**Application method**	**Parameter(s) tested for adults (Ad) or larvae (L) in lab or field**	**Abundance (Ab) and/or occurrence (O) analyses**	**Country of study**
Beynon *et al.* (2012)	Ivermectin	Pour-on	Attraction (Ad) – field (pitfall traps) Toxicity (L) – field and lab (dung pats) [a]	Ab	UK
Errouissi and Lumaret (2010)	Ivermectin	Sustained release bolus	Attraction (Ad) – field (pitfall traps)	Ab	France
Errouissi *et al.* (2001)	Ivermectin	Sustained release bolus	Toxicity (L) –lab	Ab	France
Floate (1998a)	Ivermectin	Pour-on	Attraction (Ad) – field (pitfall traps)	Ab	Canada
Floate (1998b)	Ivermectin	Pour-on	Toxicity (L) – field and lab (dung pats) [a]	Ab & O	Canada
Floate, Colwell and Fox (2002)	Ivermectin, Doramectin, Eprinomectin, Moxidectin	Pour-on	Toxicity (L) – field and lab (dung pats) [a]	Ab & O	Canada
Floate (2007)	Ivermectin, Doramectin, Eprinomectin, Moxidectin	Pour-on	Attraction (Ad) – field (pitfall traps)	Ab	Canada
Floate *et al.* (2016)	Ivermectin	Pour-on	Toxicity (L) – field and lab (dung pats) [a]	Ab & O	Canada and The Netherlands
Hempel *et al.* (2006)	Ivermectin, Moxidectin	Spiked	Toxicity (L) – lab	Ab & O	Germany
Holter, Sommer and Grønvold (1993)	Ivermectin	Injection[b]	Attraction (Ad) – field (pitfall traps)	Ab	Denmark
Holter *et al.* (1993)	Ivermectin	Injection	Attraction (Ad) – field (pitfall traps)	Ab	Denmark

Jochmann, Lipkow and Blanckenhorn (2016)	Ivermectin	Spiked	Toxicity (L) – field and lab (dung pats) [a]	Ab & O	Switzerland
Krüger and Scholtz (1998a)	Ivermectin	Injection	Toxicity (Ad) – field and lab	Ab	South Africa
Krüger and Scholtz (1998b)	Ivermectin	Injection	Toxicity (Ad) – field and lab	Ab	South Africa
Madsen *et al.* (1990)	Ivermectin	Injection	Toxicity (L) – field and lab (dung pats) [a]	Ab	Denmark
McCracken and Foster (1993)	Ivermectin	Spiked	Toxicity (Ad & L) – field	Ab & O	Scotland
Nunome, Yoshida and Niizuma (2009)	Ivermectin	Pour-on	Attraction (A) – field (pitfall traps)	Ab	Japan
O'Hea *et al.* (2010)	Ivermectin	Injection	Toxicity (Ad & L) – lab	Ab & O	Republic of Ireland
Römbke *et al.* (2007)	Ivermectin	Spiked	Toxicity (L) – lab	Ab & O	Germany
Römbke *et al.* (2010)	Ivermectin	Injection Spiked	Attraction (Ad) – field	Ab & O	Spain
Strong and Wall (1994)	Ivermectin, Moxidectin	Injection	Toxicity (Ad & L) – field	Ab & O	England
Webb *et al.* (2010)	Ivermectin, Doramectin	Pour-on	Attraction (Ad) – field (pitfall traps)	Ab & O	Scotland

[a]Dung pats exposed in field to egg-laying adults and then held in the laboratory; toxicity assessments based on counts of emergent adults

[b]Owing to lack of clarity in the method descriptions, only data collected from exposure via injection of livestock, and not through spiked dung, were used from this study in this meta-analysis.

6.3.2. Data synthesis: Occurrence

The effects of endectocides on adult beetle and larvae occurrence were tested using raw data contributed by authors (relating to n = 11 papers). Occurrence was defined as the presence of at least one individual in a given dung sample. Generalised linear mixed effects models with a binomial error structure (GLMMs; link = logit) were built in R (version 3.3.0; R Core Team 2016) using the package 'lme4' (Bates *et al.* 2015). Separate models were built for adult beetles and larvae. Study identity, species and individual dung pat identity were specified as random effects, with treatment type (control or endectocides) set as a fixed effect. Presence was coded as 1, and absence as 0. Dung-baited pitfall trap studies are best suited to assess the attraction or repulsion of residues to beetles. However, there was no material difference in the results for numbers of adult beetles when analyses were based on this subset of studies rather than the entire dataset. Therefore, to maximise statistical power, all available data were used.

6.3.3. Data synthesis: Abundance

To test the effect of endectocide exposure on the abundance of aphodiine beetles, we used the standardised mean difference (Hedges' adjusted *g*) between endectocide treated and control samples to calculate the effect size and 95% confidence interval. Hedges' *g* is a variation of Cohen's *d* that determines the post-test difference in means between two treatments. The mean difference is then divided by the pooled standard deviation to correct for small sample bias (Hedges & Olkin 1985; Hedges & Vevea 1996). Effect sizes can cautiously be interpreted as small (0.2), medium (0.5), or large (0.8 or greater) (Cohen 1988). Analyses were performed using Comprehensive Meta-Analysis (version 3.3.070, Biostat, Englewood, NJ).

Due to random errors within studies, and the variation between studies, we expected high heterogeneity and therefore chose *a priori* to apply random-effects models (REM) as the most appropriate method to calculate mean effect-size. In addition, REMs are more applicable when the aim is to generalise beyond the scope of solely those studies used in the meta-analysis (Hedges & Vevea 1998). To determine the level of heterogeneity between studies, we calculated

I^2 (level of heterogeneity as a percentage) and then tested whether the level of heterogeneity was significant using Cochran's heterogeneity statistic (Q). Higgins *et al.* (2003) tentatively assign categories of low, medium and high heterogeneity to I^2 values of 25%, 50%, and 75%. The sensitivity of the results to the exclusion of individual studies was tested using a sequential leave-one-out approach.

To permit the inclusion of data for studies where the SD of some treatment groups was zero (i.e., no traps recovered beetles), a small value (0.001) was substituted for zero. There were no material differences in the results when the analyses were repeated using the averaged SD obtained across all treatment groups within a particular study. Within this meta-analysis, the relative sample sizes for each study were weighted according to the number of dung pats examined and the number of years over which the experiments were conducted. Additionally, the number of exposure days and individual species identities were also incorporated within this analysis, to account for random variation that might occur within and between studies, and to enable a generalised meta-analysis to be performed. If a study did not report results for species individually, then the species was recorded as 'aphodiine species'.

Analyses were first performed on all studies combined, to assess the overall effects of endectocides on dung beetle abundance. Additional analyses considered outcomes for adult beetles and larvae separately. For these analyses, beetles were defined as adult individuals that had colonized fresh dung pats naturally. In contrast, larvae were immature individuals that either had been directly placed into dung by the researcher or which had developed from eggs laid in dung colonized by adults; i.e., 'progeny' (Floate 1998b).

The initial analysis used data for all endectocides combined (i.e., ivermectin, doramectin, eprinomectin, moxidectin). Each endectocide was then assessed individually when data were available for at least two studies; using this criteria both doramectin and eprinomectin were not examined for larvae as no data were available. The interaction between formulations (i.e.,

injectable (in cattle), pour-on, spiked) and experiment type (i.e., field, laboratory) was also assessed for all models. Insufficient data prevented analyses of these interactions for: i) adults in laboratory conditions, ii) adults under field conditions using spiked dung, iii) larvae under laboratory conditions using pour-on formulation, and iv) studies using a sustained release bolus formulation.

6.3.4. Publication bias

We explored the possibility of publication bias for the overall analysis of the impact of endectocides on the abundance of aphodiine beetles. Two methods were used: (1) construction of a funnel plot (Sterne & Egger 2001) and (2) the computation of the *fail-safe N* test. The former permits a visual assessment to assess whether studies with small effect sizes are underrepresented in the literature. The latter method is used to calculate the number of non-significant, unpublished studies required to nullify the overall effect size (Rosenthal 1979; Rosenthal 1984).

6.4. Results

6.4.1. Occurrence

Endectocide treated dung was significantly more likely to have at least one adult aphodiine beetle than was control dung (Odds Ratio: 1.59, CI: 1.41 – 1.79, $p < 0.001$; Figure 6.4.1). The opposite effect was found for larvae (Odds Ratio: 0.64, CI: 0.58 – 0.70, $p < 0.001$; Figure 6.4.1).

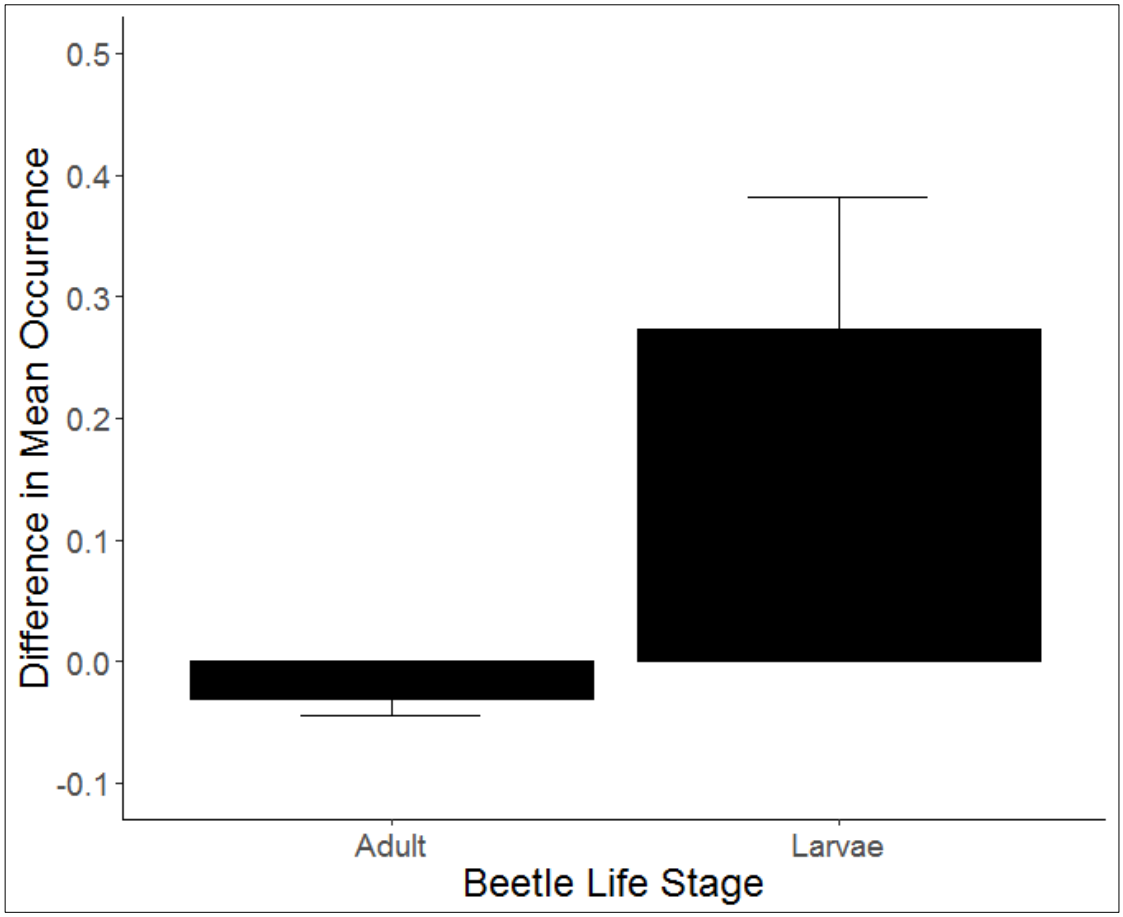


Figure 6.4.1**:** Difference in the mean presence of aphodiine beetles in endectocide-treated and control dung samples, with upper confidence intervals. Scale and direction of effect is from -1 (endectocide) to 1 (control).

6.4.2. Abundance

A significant negative relationship was detected between endectocide exposure and the total abundance of aphodiine beetles (adults plus larvae) (22 studies, Hedges' *g*: 0.46, 95% CI: 0.21 – 0.71, $p < 0.001$; Figure 6.4.2). The heterogeneity of the effect sizes among these studies was high (I^2 = 96.95%, Q = 689.60). However, sensitivity analyses showed that the exclusion of individual studies had little impact on the effect size (Appendix 6.7.3 – Appendix 6.7.5). A significant negative relationship between treatment and abundance was detected for adults (14 studies; Hedges' *g*: 0.34, 95% CI: 0.05 – 0.62, $p = 0.022$; Figure 6.4.3) and a stronger effect was detected for larvae (12 studies; Hedges' *g*: 0.52, 95% CI: 0.21 – 0.84, $p = 0.001$; Figure 6.4.4). There was high heterogeneity of effect sizes for both life stages (adults: I^2 = 93.05%, Q = 187.13; larvae: I^2

= 96.34%, Q = 301.29), but sensitivity analyses showed that the results were robust to the exclusion of individual studies (supplementary material S3).

Further analyses were conducted to assess the effect of individual endectocides on the abundance of different life stages. Ivermectin was associated with a significant negative effect on the abundance of both larvae (Hedges' *g*: 0.57, 95% CI: 0.18 – 0.86, *p* = 0.002) and adults (Hedges' *g*: 0.15, 95% CI: 0.04 – 0.62, *p* = 0.028) relative to controls. Similar negative patterns were observed for doramectin (Hedges' g: 0.30, 95% CI: -0.33 – 0.93, *p* = 0.351) and eprinomectin (Hedges' g: 0.06, 95% CI: -0.05 – 0.17, *p* = 0.281); though not significant, the results could not exclude the possibility of no effect and further research is required. For moxidectin residues, sample sizes were relatively low, and patterns consistent with either a positive or negative effect were observed for adults (Hedges' *g*: -0.19, 95% CI: -1.25 – 0.86, *p* = 0.721) and larvae (Hedges' g: 0.36, 95% CI: -1.34 – 2.05, *p* = 0.680).

Exploration of the interaction between study type (field vs. laboratory) and formulation (pour-on, injectable, spiked), showed that pour-on formulations showed a clear negative association with larval abundance in field experiments (Hedges' *g*: 0.26, 95% CI: 0.19 – 0.34, *p* < 0.001). Results for injectable formulation and spiked dung on larvae were equivocal, with more data being required to assess the direction of these effects (Injectable: Hedges' *g*: 0.49, 95% CI: -0.98 – 1.96, *p* = 0.513; spiked: Hedges' *g*: 0.32, 95% CI: -0.39 – 1.04, *p* = 0.372). In laboratory studies, a reduction in larval abundance was detected in spiked dung compared with controls (Hedges' *g*: 1.20, 95% CI: 0.65 – 1.74, *p* < 0.001). With the available data, we were unable to detect a clear positive or negative association when using injectable formulation (Hedges' *g*: 0.25, 95% CI: -0.13 – 0.64, *p* = 0.201). For adults no clear conclusion can be drawn from the field experiments using pour-on and injectable formulations (pour-on: Hedges' *g*: 0.35, 95% CI: -0.14 – 0.82, *p* = 0.160; injectable: Hedges' *g*: 0.47, 95% CI: -0.09 – 1.03, *p* = 0.100; no data were available for spiked formulations).

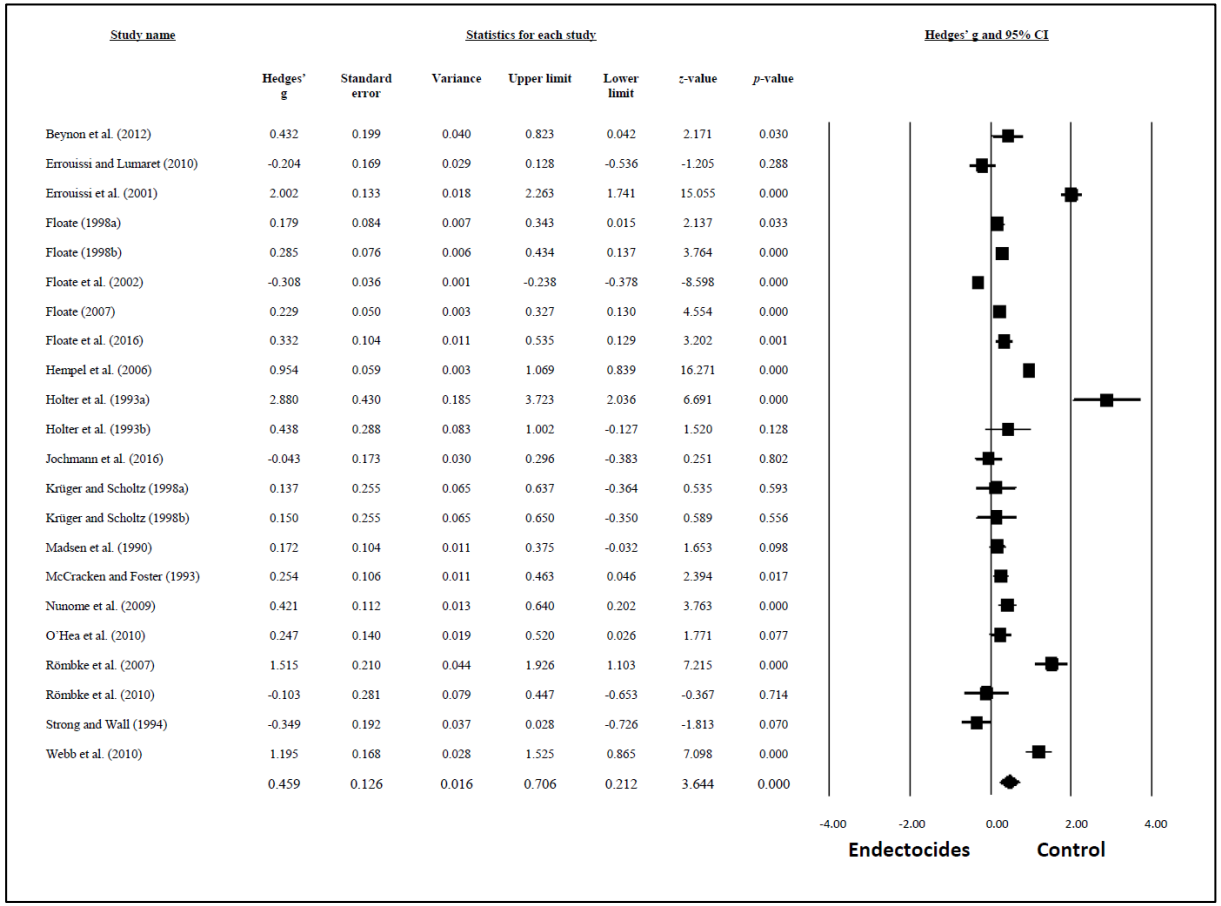


Figure 6.4.2: Forest plot illustrating the impact of endectocides on total abundance of aphodiine beetles (larvae and adults). Boxes represent Hedges' g estimates of effect size for individual studies within the overall meta-analysis, and the lines represent their 95% confidence intervals (CI). The diamond represents the combined mean Hedges' g estimate of all studies, with its width representing its 95% CI. If an effect size is positive (to the right of zero), the data has greater association with 'Control' dung rather than those exposed to 'Endectocides' (negative; to the left of zero). Thus, highlighting the direction of the effect for each study.

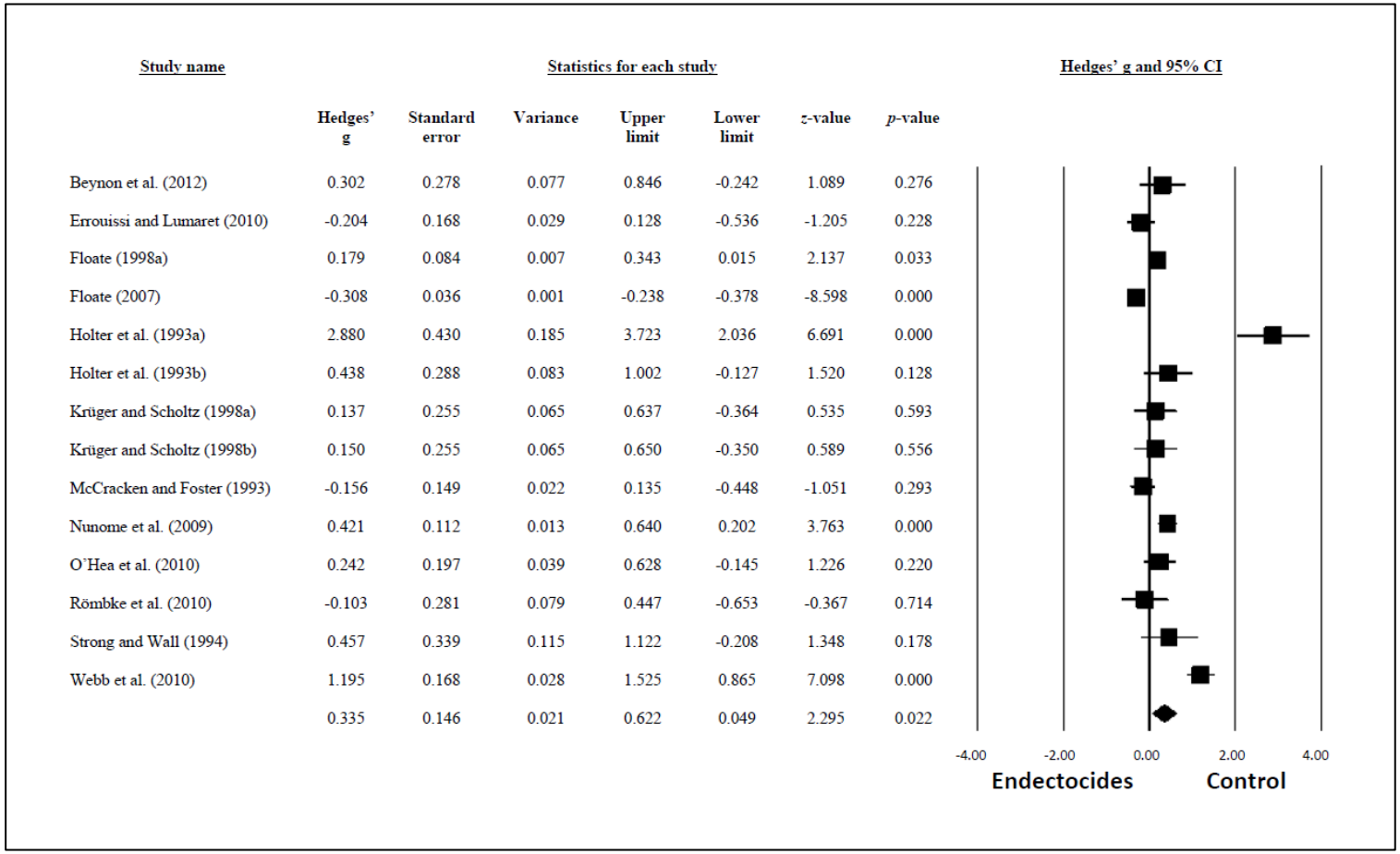


Figure 6.4.3**:** Forest plot illustrating the impact of endectocides on adult aphodiine beetle abundance. Boxes represent Hedges' g estimates of effect size for individual studies within the overall meta-analysis, and the lines represent their 95% confidence intervals (CI). The diamond represents the combined mean Hedges' g estimate of all studies, with its width representing its 95% CI. If an effect size is positive (to the right of zero), the data has greater association with 'Control' dung rather than those exposed to 'Endectocides' (negative; to the left of zero). Thus, highlighting the direction of the effect for each study.

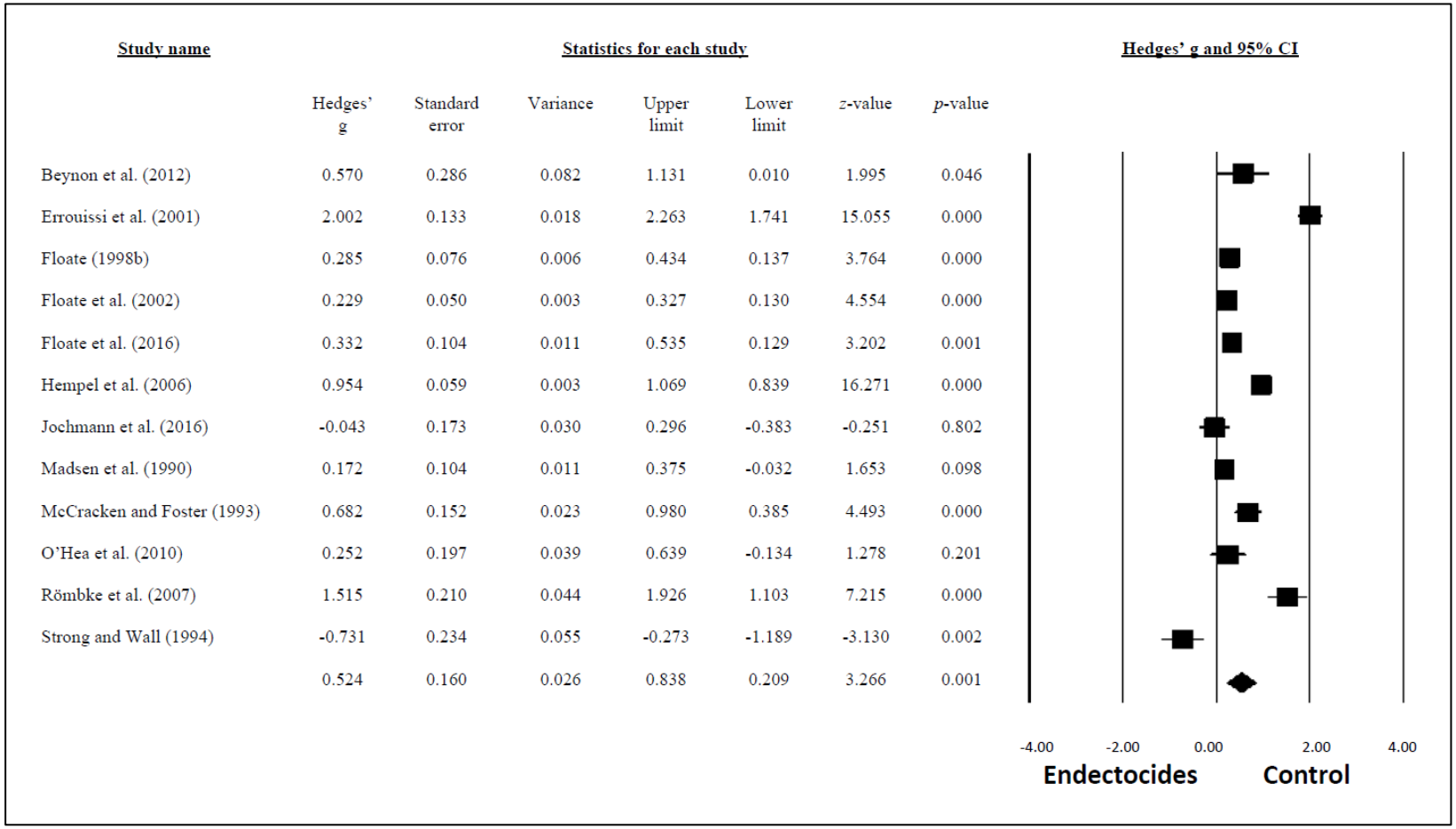


Figure 6.4.4: Forest plot illustrating the impact of endectocides on larval aphodiine beetle abundance. Boxes represent Hedges' g estimates of effect size for individual studies within the overall meta-analysis, and the lines represent their 95% confidence intervals (CI). The diamond represents the combined mean Hedges' g estimate of all studies, with its width representing its 95% CI. If an effect size is positive (to the right of zero), the data has greater association with 'Control' dung rather than those exposed to 'Endectocides' (negative; to the left of zero). Thus, highlighting the direction of the effect for each study.

6.4.3. Publication bias

The asymmetry of the funnel plot computed for the total analysis suggested the presence of small-study bias or unexplained heterogeneity (Figure 6.4.5). It was calculated that correcting for this asymmetry would require four studies (black dots in Figure 6.4.5) to fall on the right of the mean effect size, i.e. studies which show significant positive effects of endectocides on aphodiine beetles. Using an REM including the imputed values for these four missing studies, we demonstrated that the new mean effect size for the symmetrical total analysis is very similar to the original estimate (Hedges' *g* = 0.54, 95% CI: 0.31 – 0.93 compared with the original: Hedges' *g*: 0.46, 95% CI: 0.21 – 0.71), suggesting that publication bias is unlikely to explain the results. Using the *fail-safe N* method for the total analysis, 1188 additional unpublished or undiscovered studies would be required to nullify the results. Rosenthal (1984) states that effect sizes are robust if the *fail-safe N* number is five-fold greater than the number of studies used in the meta-analysis plus 10. Thus, for every dataset used in the present study, an additional 54 datasets showing no effect of endectocide residues would be needed to counter the effect of our findings. It can therefore be concluded that the estimated effect sizes in the meta-analysis, are robust and unbiased, can be interpreted in a meaningful way.

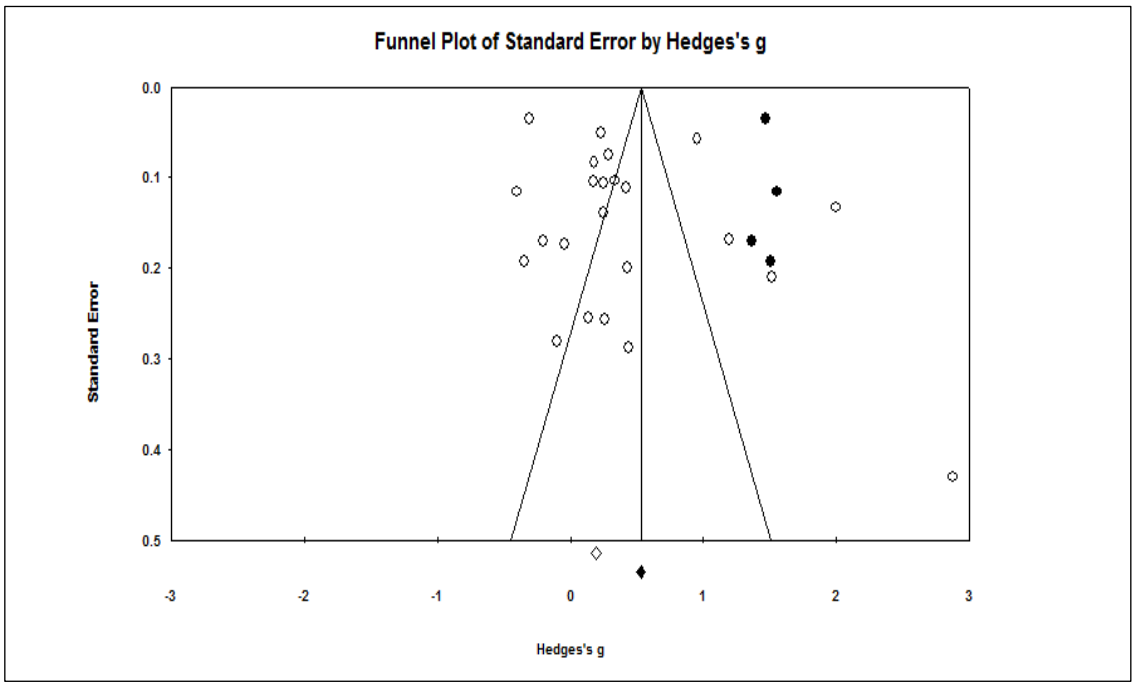


Figure 6.4.5: Hollow circles in the funnel plot represent individual studies from the total analysis (n = 22). Black circles represent imputed studies from Trim and Fill method, and the black diamond represent the 95% confidence intervals for the meta-analysis around random effect models mean adjusted for publication bias (black straight line). See text for further explanation.

6.5. Discussion

Our results indicate a significant overall negative effect of endectocide faecal residues on the abundance of both larval and adult aphodiine beetles. The high heterogeneity (I^2) associated with study-specific factors (e.g., time of year, temperature, species, endectocide product, formulation) confirm the value of developing a standardised procedure for Tier B testing (Jochmann *et al.* 2011).

The outcomes from this meta-analysis resolve the conflict between studies showing higher abundance of certain aphodiine beetles species in treatment dung (e.g. Errouissi & Lumaret 2010; Webb *et al.* 2010; Jochmann, Lipkow & Blanckenhorn 2016) and those that show the opposite effect (e.g. Floate, Colwell & Fox 2002; Floate 2007). Our results show that endectocides lower the abundance of aphodiine beetles with the effect size being larger for larvae than for adults. Hence, even if adult dung beetles are observed in dung contaminated with endectocide residues, the survival of offspring developing in that dung is significantly

reduced compared to untreated dung. Ivermectin was determined to be particularly toxic, but consistent negative patterns were also detected for the other endectocides considered. The exception was the combination of adult beetles and moxidectin, though the data were limited and are consistent with the possibility of a negative effect. However, recent research has illustrated that moxidectin did not impact adult survival or reproductive success but did impact larvae survival rates (Martínez *et al.* 2018). Confounding factors across studies could also influence these results, including variation in dose and length of exposure. It is therefore appropriate to apply a precautionary principle until further data become available, this is particularly true for all endectocides other than ivermectin. Importantly, all of the endectocides tested were linked with some form of negative impact, so it would be unwise to classify any as environmentally safe based on current evidence. It would be valuable for future research to assess other veterinary parasiticides that may have a more limited impact on the environment.

Investigating the interaction between formulation and study type (field *v.s.* laboratory) identified a significant negative impact of pour-on formulations on beetle larvae in the field, whereas evidence for the other application methods was more equivocal. Laboratory experiments only showed a reduction in the abundance of larvae developing in spiked dung, but most applications of endectocides have negative associations with beetle abundance (e.g. Krüger & Scholtz 1998b; Errouissi *et al.* 2001). However, we caution that there is a lack of studies that directly compare the non-target effects of different formulations, and this evidence gap should be filled as a matter of urgency. For example, we were unable to obtain data that directly compared in the same study, the effect of spiked dung versus dung from treated animals. There was also a lack of data assessing the abundance of adult beetles in spiked dung in field studies, and for larvae developing in dung from treated animals in laboratory studies.

Analysis of the raw occurrence data (11 papers), demonstrated that treated dung had a slightly higher probability of containing at least one adult beetle compared to untreated dung, indicating

that residues can act as an attractant. The opposite pattern was detected for larvae, suggesting that residues increase egg and larval mortality. To our knowledge, this is the first report of how the occurrence of adult and larvae aphodiine beetles is affected by endectocide residues. It highlights the potential for a 'snowball effect', whereby attraction to residues may increase the likelihood of adults laying their eggs in dung that is particularly toxic to their progeny. In the absence of immigration, the application of endectocides could therefore potentially contribute to the local extirpation of aphodiine populations. The attraction of dung beetles to residues has been reported previously, with variation within and among studies associated with year, season (e.g., spring vs. autumn) and length of exposure (e.g. Lumaret *et al.* 1993; Floate 1998a; Verdú *et al.* 2018; Rodríguez-Vivas *et al.* 2019). Römbke *et al.* (2010) state that the attraction can occur when acetone is used as a solvent in studies that use dung spiked with ivermectin. However, we observed the same effects with alternative application formulations. These results demonstrate the complexity of the issue of attraction behind individual studies, and the local factors that need to be accounted for. When investigating the effects of endectocides, more research is need on the occurrence of species and not just on their abundance.

Overall, our results clearly demonstrate the negative impact of endectocide residues on aphodiine beetles. We stress that a standardised methodological approach should be taken when conducting multi-species environmental impact assessments of different endectocide products; e.g., Jochmann *et al.* (2011); Floate *et al.* (2016); Jochmann, Lipkow and Blanckenhorn (2016). Critically, integrated research is needed to understand the synergies and trade-offs between veterinary pharmaceutical use and the delivery of ecosystem services, such as dung removal from pasture. As well as benefitting wildlife, more measured use of veterinary pharmaceuticals will slow the world-wide development of parasiticide resistance by target species. In Europe, nematodes on 12.5% of farms surveyed in four major cattle markets were recently found to be resistant to both ivermectin and moxidectin (Geurden *et al.* 2015). In Brazil, a study of 10 farms demonstrated that none of four avermectins (doramectin, eprinomectin,

ivermectin, moxidectin) were effective for the control of nematodes affecting cattle (Ramos *et al.* 2016). In the UK, guidelines have been created to manage for parasiticide resistance (Control of Worms Sustainably 2017; Sustainable Control of Parasites in Sheep 2017), however, dissemination and application of this information can be variable. Adhering to such guidelines and using parasiticide products with limited non-target effects may slow current declines being reported for insect populations (e.g. Hallmann *et al.* 2017) and will help sustain ecosystem services that annually return many millions to the global agricultural industry (e.g. Beynon, Wainwright & Christie 2015).

6.6. Acknowledgements

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6.7. Appendices

Appendix 6.7.1: Search terms used in the meta-analysis.

TS = (anthelminthic*OR anthelmintic* OR Endectocide OR Macrocyclic lactone OR Milbemycin oxime* OR Avermectin* OR MLs OR Organophosphate OR veterinary medical products OR Synthetic pyrethroid* OR salicylanilide* OR substituted phenol* OR tetrahydropyrimidine* OR imidazothiazole* OR benzimidazole* OR Ivermectin OR moxidectin OR abamectin OR eprinomectin OR doramectin) AND TS = (cattle OR cow OR sheep OR livestock) AND TS = (Aphodius OR Aphodiinae OR Aphodiidae OR Aphodius ater OR Aphodius rufipes OR scarabid* OR Scarabaeidae OR night-flying dung beetle OR dung beetle* Or Aphodius bimaculatus OR Third-instar Larva OR Aphodius borealis OR Aphodius brevis OR Aphodius coenosus OR Aphodius constans OR Aphodius conspurcatus OR Aphodius contaminatus OR Aphodius testudinarius OR Aphodius depressus OR Aphodius distinctus OR Aphodius equestris OR Aphodius erraticus OR Aphodius pedellus OR Aphodius fimetarius OR Aphodius sylvestris OR Aphodius foetens OR Aphodius foetidus OR Aphodius fossor OR Aphodius frater OR Aphodius gissaricus OR Aphodius granarius OR Aphodius haemorrhoidalis OR Aphodius ictericus OR Aphodius immundus OR Aphodius lapponum OR Aphodius lividus OR Aphodius lugens OR Aphodius luridus OR Aphodius rufa OR Aphodius melanostictus OR Aphodius merdarius OR Aphodius nemoralis OR Aphodius villosus OR Aphodius niger OR Aphodius sus OR Aphodius obliteratus OR Aphodius obscurus OR Aphodius paykulli OR Aphodius piceus OR Aphodius pictus OR Aphodius plagiatus OR Aphodius porcus OR Aphodius prodromus OR Aphodius punctatosulcatus OR Aphodius punctipennis OR Aphodius pusillus OR Aphodius putridus OR Aphodius quadriguttatus OR Aphodius quadrimaculatus OR Aphodius rufipes OR Aphodius rufus OR Aphodius scrofa OR Aphodius serotinus OR Aphodius sordidus OR Aphodius consputus OR Aphodius sphacelatus OR Aphodius subterraneus OR Aphodius tomentosus OR Aphodius uliginosus OR Aphodius fasciatus OR Aphodius arenarius OR Aphodius varians OR Aphodius zenkeri OR Aphodius *sticticus*)

Appendix 6.7.2: Table providing additional information on the cattle breeds, their diet and dosage the endectocide used in each study.

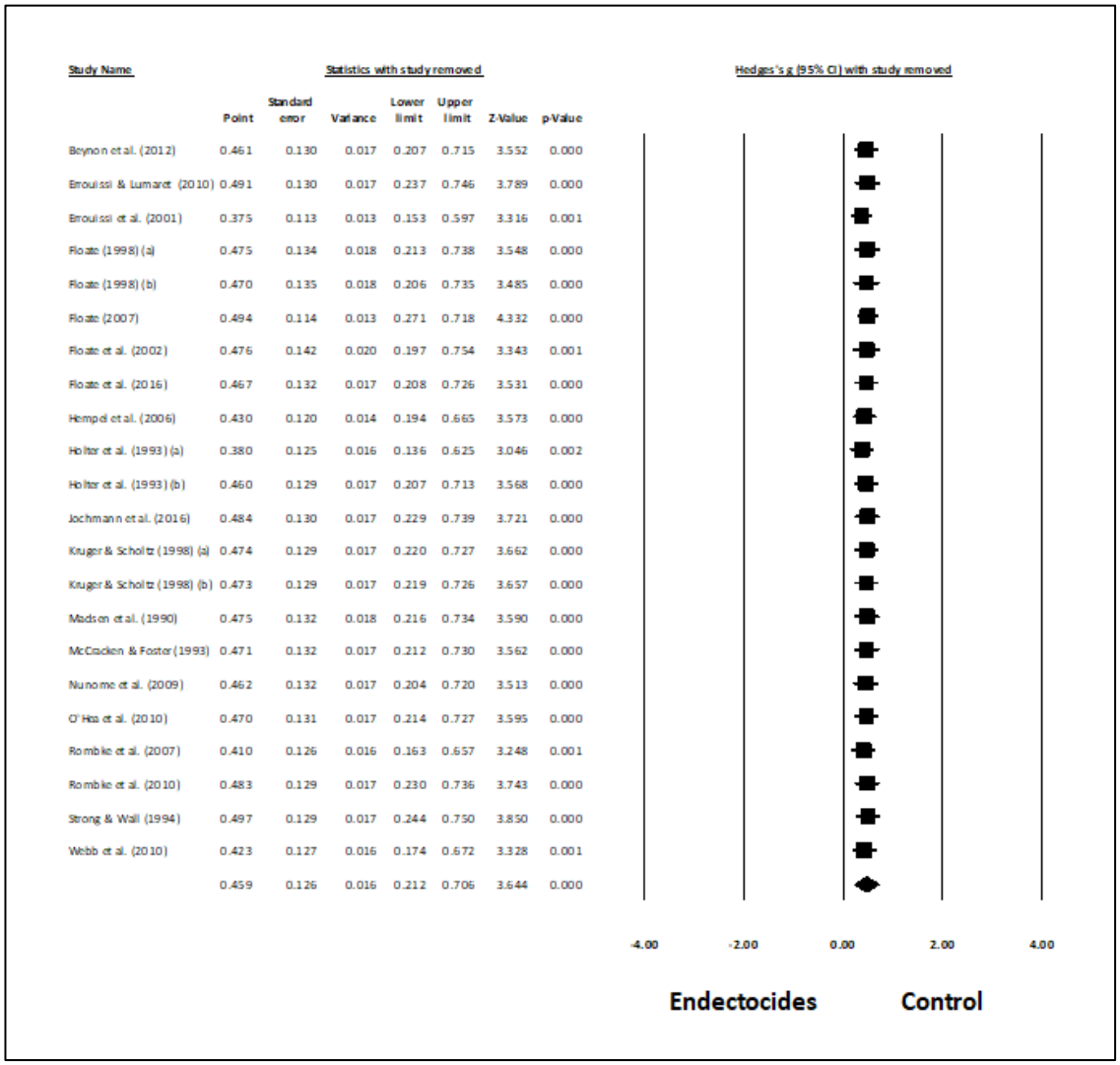
Source of data	**Endectocide**	**Application method**	**Cattle Breed**	**Cattle Diet**	**Application Dose**
Beynon *et al.* (2012)	Ivermectin	Pour-on	Holstein friesian	Whole wheat and peas	Animec® 10 ml/kg
Errouissi and Lumaret (2010)	Ivermectin	Sustained release bolus	Aubrac heifers (weighing between 100 and 300 kg)	Hay	Ivomec® SR bolus 12 mg/day
Errouissi *et al.* (2001)	Ivermectin	Sustained release bolus	Charolais steers and aubrac heifers (weighing between 150 and 450 kg)	Hay	Ivomec® SR bolus 12 mg/day
Floate (1998a)	Ivermectin	Pour-on	No breed specified (460 kg average body weight)	Alfalfa cubes and barley silage	500 mg/kg body weight Ivomec®
Floate (1998b)	Ivermectin	Pour-on	Steers and heifers (no breed specified; 460 kg average body weight)	Alfalfa cubes and barley silage	500 mg/kg body weight Ivomec®
Floate, Colwell and Fox (2002)	Ivermectin, Doramectin, Eprinomectin, Moxidectin	Pour-on	Group 1: heifers (no breed specified; 206 kg average body weight); Group 2: steers (no breed specified; 370 kg average body weight); Group 3: steers (no breed specified; 375 kg average body weight)	Group 1: barley silage and rolled barley; Group 2: barley silage, chopped alfalfa, alfalfa silage and rye silage; Group 3: barley silage, chopped alfalfa, rolled oats and barley, and early lactation grain ration	500 µg kg^{-1} body weight
Floate (2007)	Ivermectin, Doramectin, Eprinomectin, Moxidectin	Pour-on	Group 1: steers (no breed specified; 300 kg average body weight); Group 2: steers (no breed specified; 347 kg average body weight); Group 3: steers (no breed specified; 375 kg average body weight)	NA	500 g/kg body weight

Floate *et al.* (2016)	Ivermectin	Pour-on	Group 1: Aubrac heifers (361 kg average body weight); Group 2: Holstein steers (558 kg average body weight); Group 3: Holstein cows (1077 kg average body weight)	Group 1: hay and grass; Group 2: hay; Group 3: barley silage	500 µg kg body weight Ivomec®
Hempel *et al.* (2006)	Ivermectin, Moxidectin	Spiked	NA	Grass and hay	0.1, 1, 10, 100, mg a.s./kg dung
Holter, Sommer and Grønvold (1993)	Ivermectin	Injection	Heifers (no breed specified; weighing between 150 and 450 kg)	NA	0.2 mg kg^{-1} body weight
Holter *et al.* (1993)	Ivermectin	Injection	Red Dane/Friesian cross (weighing between 150 and 450 kg)	NA	0.2 mg kg^{-1} body weight
Jochmann, Lipkow and Blanckenhorn (2016)	Ivermectin	Spiked	NA	NA	65.67, 20.75, 6.57, 2.08, 0.657, and 0.208 mg/kg fresh dung
Krüger and Scholtz (1998a)	Ivermectin	Injection	NA	NA	200 µg kg^{-1} body weight
Krüger and Scholtz (1998b)	Ivermectin	Injection	NA	NA	200 µg kg^{-1} body weight
Madsen *et al.* (1990)	Ivermectin	Injection	Red Dane heifers (c300 kg body weight)	NA	500 µg kg^{-1} body weight Ivomec®
McCracken and Foster (1993)	Ivermectin	Spiked	NA	NA	0.5, 1, and 2 mg/kg dung

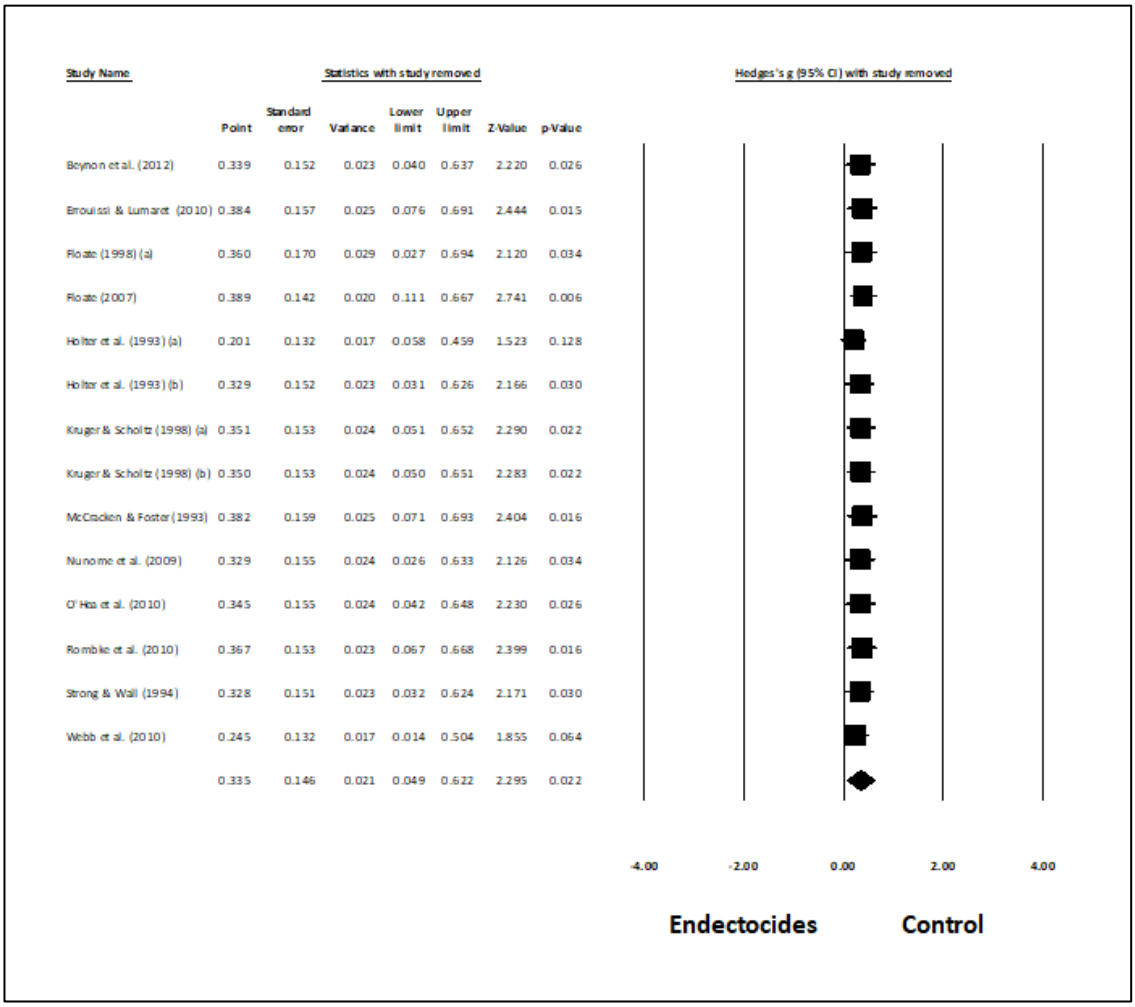
Nunome, Yoshida and Niizuma (2009)	Ivermectin	Pour-on	Group 1: japanese black cattle; Group 2: holsteins and jerseys	Group 1: grass and alfalfa; Group 2: Hay, berets, grass	NA*
O'Hea *et al.* (2010)	Ivermectin	Injection	NA	Grass	0.2 mg kg^{-1} body weight Qualimec™
Römbke *et al.* (2007)	Ivermectin	Spiked	NA	Grass and hay	0.1, 1, 10, 100, 1000 mg a.s./kg dung
Römbke *et al.* (2010)	Ivermectin	Injection Spiked	Holstein cross (weighing between 480 and 580 kg)	Hay	200 µg kg^{-1} body weight and 10.8 mg kg^{-1} dung Ivomec®
Strong and Wall (1994)	Ivermectin, Moxidectin	Injection	Limousin/fresian cross and belgium blue/fresian cross (302.7 kg average body weight)	Silage	200 µg kg body weight Ivomec®
Webb *et al.* (2010)	Ivermectin, Doramectin	Pour-on	NA	Grass	500 µg kg body weight Ivomec® and Dectomax™

*no doseage of Ivermectin was reported in the paper.

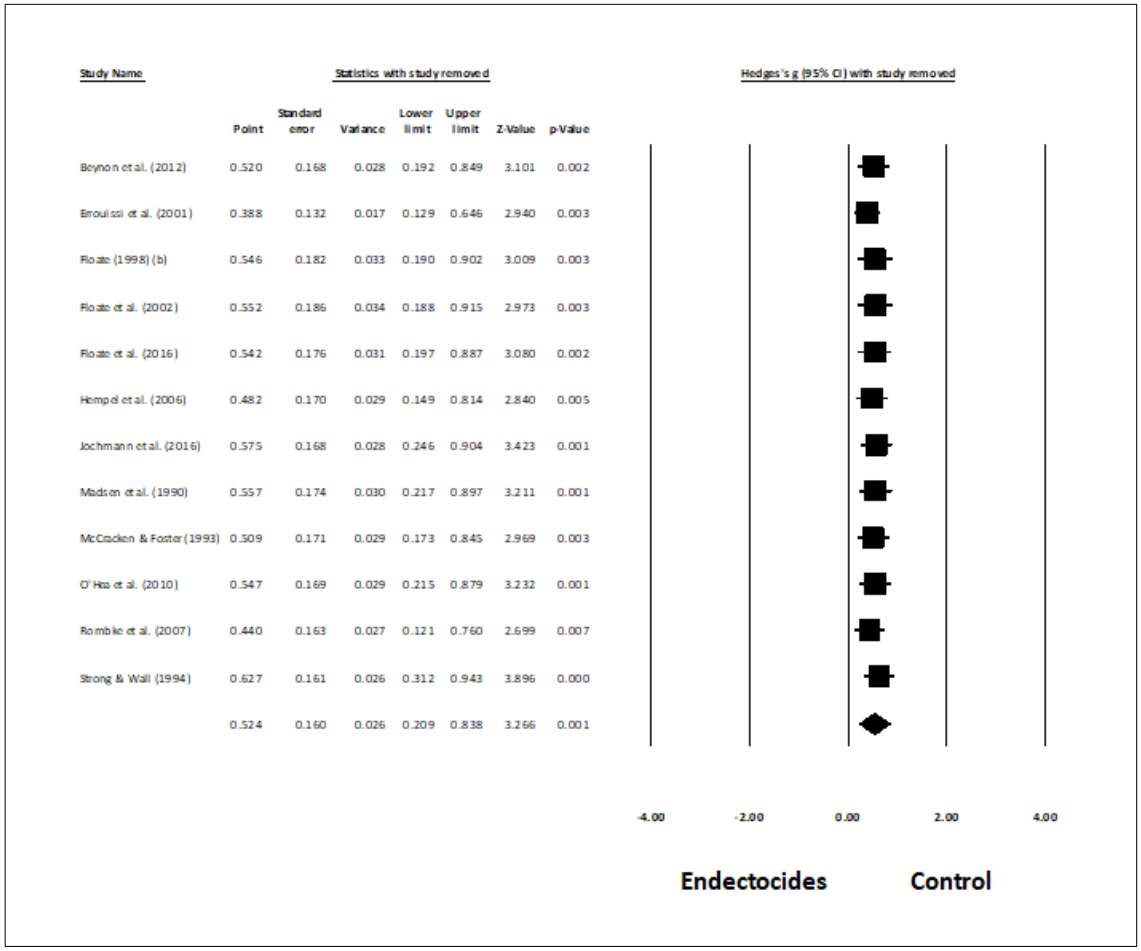
Appendix 6.7.3: Sensitivity analysis of cumulative analysis (one study removed).



Appendix 6.7.4: Sensitivity analysis of adult data (one study removed).



Appendix 6.7.5: Sensitivity analysis or larvae data (one study removed).



Chapter 7
General Discussion

7.1. Overview

Urbanisation and the intensification of agricultural land management are known to have significant negative effects on the environment — such as habitat fragmentation and reduction in prey availability. These changes have the potential to not only affect the abundance of species, but also the social structure of entire populations. Effective monitoring of how these negative anthropogenic pressures impact species allows evidence-based conservation and practical management strategies to be developed. In this thesis, I have combined computer-modelling approaches, a meta-analysis, and field experiments, to understand how landscape features and land management practices might influence the movement patterns and relative abundance of *Rhinolophus ferrumequinum* across anthropogenically altered landscapes.

7.2. Summary of results

Most of Britain's landscapes are highly modified, with centuries of anthropogenic pressures shaping and reshaping the landscape. As a result, habitats with high value to wildlife, such as hedgerows connecting ancient woodland patches, can often be found within wider areas of relatively low ecological value. Urbanisation, as well as changes to the agricultural landscape, have been shown to cause fragmentation at a landscape scale, creating large areas of low ecological value. To date there has been important work on the overall impacts of roads and lights on abundance and diversity of bat species (Stone, Jones & Harris 2009; Berthinussen & Altringham 2012b; Stone, Jones & Harris 2012; Rowse *et al.* 2016; Rowse, Harris & Jones 2016; Pourshoushtari *et al.* 2018; Claireau *et al.* 2019b; Medinas *et al.* 2019), but very little research on other aspects of road ecology (e.g. traffic noise). In Chapter 2, I provide novel insight into the effects of traffic noise on bat activity. Using BACI field experiments over two field seasons (firstly identifying the overall impact of traffic noise, and secondly examining the potential separate impacts of sonic and ultrasonic spectrums), I showed that traffic noise negatively impacts the relative activity for five bat species (or species groups), and the feeding behaviour of two species. My research shows that the sonic spectrum (0-20 kHz) has a larger negative impact compared

to the ultrasonic spectrum (>20 kHz). This was true across all of bat species examined, despite notable different in flight heights, speeds, foraging strategies, and echolocation patterns. The results suggest that the responses of bats to traffic noise are likely to be a generalised phenomenon that has a negative impact across different functional groups. The mode of action is therefore likely to be through general deterrence and avoidance, rather than through the masking of echolocation calls used for orientation or foraging, concurring with the results of laboratory-based studies (e.g. Luo, Siemers & Koselj 2015).

The results from my third chapter illustrate the importance of other types of linear features within the environment, which can act as foraging grounds and commuting routes for bats within landscapes dominated by the less suitable habitat. Treelines were found to have significantly higher bat activity for two species, *P. pygmaeus* and *R. ferrumequinum*, compared to any other type of linear feature. Overall, these results contribute to our current scientific understanding of how bats utilise the agricultural environment, highlight which features and management regimes are beneficial to increasing species activity and connecting them with the wider environment (MacDonald & Johnson 1995; Staley *et al.* 2012; Froidevaux *et al.* 2019; Froidevaux, Broyles & Jones 2019). However, an important novel finding from my research was that about a third of all *R. ferrumequinum* activity recorded at paired detectors was derived from the middle of fields. This information is particularly useful when conducting Environmental Impact Assessments for new developments in open agricultural environments.

From my research – shown in Chapters 2 and 3 – and the current literature, it is known that some linear features can increase permeability of the wider landscape for *R. ferrumequinum*, whereas other such as traffic noise, light and roads can cause avoidance behaviours in bats, acting as barriers to species movement. However, understanding the cumulative impact of these pressures on species of conservation concern is technically challenging — despite the necessity of incorporating such cumulative assessments in Ecological Impact Assessments (Environmental

Impact Assessment (EIA) Directive (2014/52/EU)). In Chapter 4, I addressed this issue using a novel predictive computer modelling technique to examine the functional connectivity of landscape features for *R. ferrumequinum*. Using non-invasive static bat detectors as a method of ground validation, I show how robust models relating to species movement can be made, highlighting routes of high functional connectivity and points that play important roles in linking *R. ferrumequinum* roosts to potential foraging grounds in the wider environment. The results highlighted the significant negative impact street lights can have on the movement of *R. ferrumequinum*, but also identify locations where practical conservation measures can be applied to potentially alleviate negative pressures and allow greater permeability for species.

In addition to ecological factors, attributes of individual animals, such as their age, sex, and breeding status, can influence the way in which bats are spatially distributed. As explained in Chapter 5, social structure exists within hibernating *R. ferrumequinum*. Using a 17-year dataset, I show that adult males are more central within the social network during the hibernation period; whether this is for information-sharing, mating purposes or could potentially cause disease transmission. This is a stark contrast to *R. ferrumequinum* roosting ecology during the maternity season, were females usually cluster together and have little interactions with males (Ransome 1968; Ransome & Hutson 2000). I found that movements between sites were associated with both age and degree centrality of individual bats, with less-central hibernacula playing an important role for the movement of certain individuals. This highlights that despite low activity in some of these smaller roost locations, they are a conservation concern to decrease the risk of fragmentation and loss of connectivity within the wider landscape. This research identifies the need for understanding year-round patterns of species interactions and movement, which are essential for developing realistic species management plans.

The activity levels of *R. ferrumequinum* within the environment may not only be influenced by the type physical management which is applied, e.g. ploughing and grazing fields or trimming

hedgerows, but also by the application of additional treatments such as fertilisers, insecticides, pesticides and endectocides to increase productivity. Endectocides are used to prevent or reduce parasitic loads within livestock but have the potential to suppress dung-associated invertebrate populations substantially and, in turn, the availability of prey for *R. ferrumequinum*. This is important as approximately 33% of their diet consist of Coleoptera and of those 29% were beetles that are associated with cow dung, primarily *Aphodius spp.* (Jones 1990). However, research in this area to date have presented contrasting results. To clarify this situation, I conducted a meta-analysis on all the available studies (n = 22), which examined the impact of endectocides on both the abundance and occurrence of adult and larvae aphodiine dung beetles. The results demonstrated that endectocides, particularly ivermectin, had significant negative effects on both adult and larvae dung beetles. The results of the meta-analysis, using data from field experiments only, also provides evidence of the toxicity of pour-on formulations on Aphodiine abundance; and insufficient evidence to effectively assess whether the alternatives are safe. Conversely, dung obtained from cattle treated with endectocides has higher occurrence rates of dung beetles than controls – indicating attraction – whereas the occurrence and abundance of larvae is reduced, presumably because of toxicity. Overall, this meta-analysis highlights how farming practices, e.g. parasite management in livestock, if not managed appropriately, can have significant negative effects on invertebrate populations. Not only reducing prey items for *R. ferrumequinum* and other species but also considerably impacting the ecosystem service that dung beetles provide, such as nutrient recycling, increasing soil aeration, and reducing pest species (Bornemissza & Williams 1970; Nichols *et al.* 2008; Beynon *et al.* 2012; Beynon, Wainwright & Christie 2015).

7.3. Recommendation

Under the current EU legislation (Habitats Directive), member states have to implement specific conservation measures to achieve favourable conservation status for annex species (*R. ferrumequinum* are both an Annex II and Annex IV species). For such species, it is not only their

resting places (i.e. roosting, nesting or denning locations) and foraging grounds that need to be considered when assessing potential impacts but also the wider environment in order to maintain the integrity of these places. To achieve this status, a landscape scale approach may need to be taken with consideration given to meta-population dynamics, social structure, heterogeneity and connectivity within the landscape. This not only takes into account the composition of the landscape and the amount of any ecological resource, e.g. woodlands, but also the configuration and the spatial arrangement of the features within it.

Based on the results of my research and the relevant published scientific literature, the following recommendations are made to reduce and mitigate the impacts of habitat fragmentation and changes to land management at both a local and landscape scale. While these recommendations are largely focused on *R. ferrumequinum*, they are applicable to other species.

7.3.1. Agricultural environments

Hedgerows act as boundaries between adjacent lands, store carbon and regulate the water table (Holden *et al.* 2019); but they also act as critical habitats for species to breed, forage and commute within agricultural environments. My results showed that those hedgerows managed sympathetically are more likely to have greater bat activity compared to those managed intensively (Chapter 3). In addition to my research, the literature highlights that having linear features with taller vegetation and trees, as well as the total length of hedgerows in the wider environment (Verboom & Huitema 1997), can increase bat activity. In addition, Pinaud *et al.* (2018) showed that *R. ferrumequinum* are less likely to cross hedgerows/woodlands habitats that have gaps larger than 38 metres. These results show the need to increase connectivity of linear features within the landscape to prevent the isolation of resources such as foraging areas. Such conservation measures could include, only trimming the hedgerow every three years, cut the hedgerows on rotation between field boundaries and not managing them intensively (Staley *et al.* 2012; Froidevaux *et al.* 2019; Froidevaux, Broyles & Jones 2019).

Attempts have been made to implement increased connectivity and biodiversity benefits into agri-environmental schemes (AES), but with limited resources for ensuring compliance or implementation, the success of these measures are largely unknown (Kleijn & Sutherland 2003). The current model for protecting biodiversity within agricultural settings has a number of limitations at a landscape scale and a shift in policy of these interventions, from farm-based payments to result based payments within new Environmental Land Management schemes (public money for public goods), is required (Haaren & Bathke 2008; Armsworth *et al.* 2012). In addition, little work has been done in achieving spatially targeted and coordinated approaches to AES. Such approaches could utilise the work carried out in Chapter 4, to ensure that the restoration or creation of new habitat could be implemented in the best locations to achieve the highest net gain for biodiversity, as well as the greatest benefit for landowners alike. Spatially targeted approaches have been shown to be more likely to yield higher biodiversity benefits when compared to non-spatially targeted works. For example, the farmland bird species cirl bunting and the stone curlew (Bealey *et al.* 1999; Green, Tyler & Bowden 2000; Stanbury *et al.* 2010; Davies *et al.* 2011) achieved local recovery because spatially target conservation measures were applied. These results suggest that enhancing or maintaining landscape heterogeneity and spatially targeting resources are likely to be highly effective methods of conserving biodiversity within agricultural landscapes (Concepción, Díaz & Baquero 2008). This is particularly relevant for *R. ferrumequinum*, as Froidevaux *et al.* (2017) shows that the size of colonies are significantly correlated to the availability of resources surrounding each roost.

To achieve these outcomes, toolboxes such as Circuitscape (Chapter 4) and habitat suitability models could be used. These can identify the best location to create new hedgerows allowing wider connectivity in the landscape, which agricultural fields to afforest for increasing foraging and roosting habitat for bats, and to identify those areas that already act as critical corridors for species movement and to protect them from further anthropogenic pressures.

For *R. ferrumequinum*, it is critical that both a landscape scale and a multi-season approach is taken for their conservation. Previous research has shown that *R. ferrumequinum* are active and forage throughout the hibernation period (Park, Jones & Ransome 1999; Park, Jones & Ransome 2000). This means that conservation measures will have stronger positive effects if implemented in both the summer and wintering areas of the species. These measures should have special consideration for how individual attribute can influence their roosting ecology at different times of the year and, particularly, the importance of implementing these measures at smaller more isolated roosting locations in the wider network (Chapter 5).

Conservation plans not only need to consider the effects of changes and pressures to the physical environment, but also the application of veterinary products on livestock. In Chapter 6, I demonstration the significant negative effects endectocides can have on dung beetles, a key prey item of *R. ferrumequinum* during the breeding season. Simple measures to reduce the impact of endectocides on dung fauna could save the environment and the economy £40.2million year−1 (£4.36 per cow) under agri-environment schemes (Beynon, Wainwright & Christie 2015). Such measure include having mixed stock herds and rotate them over longer periods of time, leaving fields fallow for at least three weeks. In addition, only those livestock that need to be treated should be treated (whilst avoiding under-dosing), rather than the entire herd being treated at once. If livestock require treatment, those chemicals that have the lowest environmental impact should be preferential used and the treated animals should be kept indoors or off fresh pasture for at least a week, allowing large proportions of the chemical to pass through their dung.

7.3.2. Urban environments

The expansion of urban areas, and in particular roads, has been highlighted as one of the ten main threats to biodiversity (Maxwell *et al.* 2016). Roads are known to cause habitat loss, degradation and fragmentation, they act as barriers through the effect of their lighting schemes and direct mortality by collision with vehicles (Trombulak & Frissell 2000; Fensome & Mathews

2016). Their effects are not localised and can be recorded hundreds of meters away from the source location (Forman 2000; Forman & Deblinger 2000; Berthinussen & Altringham 2012b; Kitzes & Merenlender 2014). My results show (Chapter 2) that traffic noise can be added to this list, having a significant negative effect on free-living bat species. These results highlight how a single factor can contribute to the barrier effect of roads, causing the avoidance of key foraging areas and a loss of connectivity at a landscape scale for bats.

Using the predictions made from the toolboxes used to create functional connectivity models in Chapter 4, key crossing locations or pinch-points in the landscape could be identified to apply practical mitigation measures. These could include noise barriers, substrate alterations and speed limits (Wayson 1998; Ishizuka & Fujiwara 2004) but research is needed to test the effectiveness of alternative measures. New mitigation strategies are particularly needed to reduce the impact of sonic noise created by vehicles. Unfortunately, this is more difficult than mitigation for ultrasound which is readily attenuated over a shorter distance through air. Although the transition to electric vehicles may reduce road noise within urban centres, it is unlikely to have a material impact for most roads because at speeds >75km/hour, sound is generated primarily by the contact between the tyres and road surface rather than by engines (The Highway Agency *et al.* 2011). Alterations to tyre composition and structures are therefore a more promising route to reducing the impacts of traffic noise.

Other options surrounding these crossing points could be to design more permanent structures such as underpasses and overpasses, either creating them during new road schemes or retrofitting them after. These structures have the potential to create much larger corridors in the landscape, making it safer for both animals and people alike (Olsson, Widén & Larkin 2008; McGregor, Wilson & Jones 2015; Sawyer, Rodgers & Hart 2016). Bat gantries have been installed at a number of locations throughout Europe, and there is much criticism as to whether they actually provide bats with safe crossing points at roads. Berthinussen and Altringham (2012a)

demonstrates that gantries, in Britain, did not guide bats to cross the road at a safe height, despite some being on existing commuting routes. In contrast, both Claireau *et al.* (2019a) and Claireau *et al.* (2019c) found that in France, bat gantries could work as long as they were placed in the correct position (along previously known commuting routes). Underpasses have also been shown to be effective mitigation measures to increase connectivity for bats and other animals (Sawyer, Lebeau & Hart 2012; Sawyer, Rodgers & Hart 2016). Similar issues surrounding their design have been recognised, with larger underpasses, positioned in the right locations and at the correct height, having higher activity levels compared to smaller ones (Boonman 2011; Berthinussen & Altringham 2012a; Davies 2019). This identifies the need to start thinking about implementing larger green bridges at key locations, which could serve as corridors for other large mammals and not just bat species.

The current literature (Stone, Jones & Harris 2009; Falchi *et al.* 2011; Stone, Jones & Harris 2012; Day *et al.* 2015; Wakefield *et al.* 2015; Macgregor *et al.* 2017; Russo *et al.* 2017; Azam *et al.* 2018), highlights the significant negative impact lights can have on bat species, both impacting their prey items foraging potential (e.g. Lepidoptera) and movement within the landscape. My research, Chapter 4, also crucially recognises the severity of these impacts on species movement, with street lights having the highest resistance value of any environmental variable. By pinpointing the areas within the urban environment where these impacts occur (e.g. using Circuitscape), we can target practical mitigation measure to achieve the highest conservation gains. One of the simplest measures is to avoid any unnecessary lighting where possible. However, were lighting is required, all lighting should be dimmed as much as possible (Rowse, Harris & Jones 2018) and light spill above the horizontal plane should be eliminated. These lights should not be higher than required, with lower lights or bollard style lights where possible. Light baffling should be placed on all light fixtures to stop any back spill, to help reduce light trespass into important foraging and commuting areas (Falchi *et al.* 2011; Stone, Harris & Jones 2015; Voigt *et al.* 2018). Another, more recent option, has been to alternate the spectral output of LED

lights to achieve a red spectrum (reduced blue wavelength). This has been shown to increase activity of *Myotis* and *Plecotus* species when compared to white light, but a negative effect was observed for lesser horseshoe bats (*R. hipposideros*) (Spoelstra *et al.* 2015; Spoelstra *et al.* 2017; Zeale *et al.* 2018). However, there are some draw backs to this type of lighting scheme with public opposition being recorded as well as significant negative impacts on migratory bird species being observed (Gauthreaux Jr *et al.* 2006; Poot *et al.* 2008). This suggests that while lighting schemes should be designed with specific taxa in mind, consideration should be given to other taxa. This is particularly true for the prey item of many species, such as insects, which have been undergone dramatic declines in recent times (Conrad *et al.* 2006; Forister, Pelton & Black 2019).

7.4. Future research

The research presented in this thesis uses novel experimental and computational techniques to address some of the issues relating to landscape scale management of *R. ferrumequinum*. Although these techniques can generate a step-change in our approach to *R. ferrumequinum* conservation, further research is needed to address a number of issues.

7.4.1. Predictive conservation and remote sensing

Many practical conservation measures that are implemented are reactive rather than proactive, e.g. adding baffles to streetlights. In contrast, predicting likely future impacts on biodiversity is critical to our ability to design measures to future-proof the landscape, e.g. land use change or climate change (e.g. Razgour 2015). Having this foresight can allow conservation organisations to either have a management plan already prepared, or to make predictions about the severity of the impact.

The visualisations of such future projections could be tested through GIS and the use of software's like MaxEnt or Circuitscape (e.g. Razgour 2015). For example, the relative impacts of alternative scenarios, such as the positioning of new housing or lighting schemes, the creation of woodland or the restoration of hedgerows, can be assessed through this modelling process,

helping to achieve evidence-based wildlife conservation. However, to take this approach one step further is required in order to allow real-time decisions to be made: based on the premise and underlying algorithms of machine learning and artificial intelligence technologies, user-friendly web applications could be developed to allow local authorities, developers, conservation organisations and community groups to input potential new developments; viewing how these structures or lighting schemes might influence the cumulative functional connectivity of a species across a landscape, rather than just examining the impacts within the immediate vicinity of a development. This would allow the most appropriate positioning of such developments to be tested before any permanent structure is built or before any money is spent on subsidising land improvements e.g. new hedgerows being planted for bats during AES.

Similarly other technologies, such as remote sensing, could be used to obtain a more accurate understanding of how individual bats utilise and move along different features within the landscape. The application of such tools has the ability to provide information not only on species movement, in terms of GPS fixes, but their flight speeds, height, proximity to other individuals, and even foraging behaviour in some cases (Berry, Mackey & Brown 2007; Robertson & Radford 2009; Kotzerka, Garthe & Hatch 2010; Suryan, Santora & Sydeman 2012; Tew Kai *et al.* 2013; Neumann *et al.* 2015; Roeleke *et al.* 2016; Ripperger *et al.* 2020); as well as being utilised to recorded environmental variables, e.g. temperature/light/humidity sensors.

These approaches have the capability to overcome many of the difficulties encountered when trying to incorporate research into real-world decision-making by local planners (Opdam, Foppen & Vos 2002). My suggested approaches have the potential to facilitate evidence-based policy and management. The resultant outputs of the predictive models can help planners and conservationists reduce human-wildlife conflicts, by applying mitigation measures strategically at locations likely to be most sensitive to species movement and future land-use change.

7.4.2. Practical conservation and species ecology

By using predictive approaches, practical mitigation measures can be specifically designed for key pinch-point locations identified during the modelling process, particularly road crossing points.

As highlighted above, structures developed to reduce habitat fragmentation (e.g. under/overpasses) can be extremely useful in aiding species movement and increasing genetic diversity into the wider landscape (Olsson, Widén & Larkin 2008; Sawyer, Rodgers & Hart 2016); but they can also be costly, in-practical and unsuccessful. To date, very little practical research has been conducted on the effectiveness of the designs of different crossing structures, e.g. the wire designs in bat gantries, before they are permanently fixed in place. Future research now needs to focus on testing practical interventions designs to improve the safety of road crossings for bats and other species. Such measure and designs could be first scaled down to assess their effectiveness across large gaps in hedgerows, e.g. those over 38 meters for *R. ferrumequinum* (Pinaud *et al.* 2018), and then implemented at a larger scale in partnership with local road authorities.

Specific measure could be designed and tested to reduce the impact of traffic noise, principally in the sonic spectrum, on bat activity and foraging behaviour. Wildlife friendly sound barriers could be placed at key locations to alleviate some negative impacts of traffic noise and increase the permeability of those locations for bat species. However, these approaches could be limited due to the sound travelling beyond the barriers into the wider landscape and there is a question of whether drawing bats into these locations is actually beneficial. Nevertheless, their effectiveness at reducing traffic noise and whether this is actually desired for bat species needs to be investigated further. Similarly, future research could focus on using deterrents for species to decrease the risk of them crossing roads at specific locations, thus encouraging them to cross at other locations, e.g. purpose build green bridges. Some research into this field has already begun with specific asphalt being designed to emit an ultrasonic deterrent as vehicles approach

specific pinch points in the landscape, acting as an experimental audible warning system. The idea being that the early warning system only deters bats when collision risk is imminent, reducing the overall barrier effect of roads (Fourasté *et al.* 2014).

7.4.3. Analysis of social structure and movement at a landscape scale

While the research of this thesis focussed on movements around their maternity roosts (Chapter 4) and between multiple hibernation locations (Chapter 5), future research should examine the connectivity of *R. ferrumequinum* roosts and the potential impacts on their dispersal abilities across their entire population at a landscape scale. This would identify whether some meta-populations are at risk of becoming isolated, both genetically and structurally, and vulnerable to local extinction (Wright *et al.* In prepreation). Very little is understood about the larger movements of *R. ferrumequinum* during the spring and autumn, e.g. what routes both male and females use to travel between their summer and hibernation roosting locations, with most studies having focused on daily movements around maternity colonies rather than their seasonal movement patterns.

As I have shown in Chapter 5, male *R. ferrumequinum* play a key role in maintaining social links between individuals throughout the hibernation period, but male roosting behaviour during the summer months is relatively understudied. During these months males usually occur roosting in isolated locations, on their own, away from the maternity colonies, consequently, they are constantly at risk from anthropogenic pressures, e.g. home renovations. However, the relative cumulative effect of losing single non-maternity roosting locations at a landscape scale is unknown, e.g. mating roosts. I have shown (Chapter 5) that smaller roosting locations do play a role in *R. ferrumequinum* social networks during the hibernation period. Additionally, having collaborative approaches to examine what role maternal lineages, and the use of genetic techniques, play in determining social patterns during hibernation periods will provide key information for their conservation. While, in this thesis, I found no significant relationship between sex and age clustering together at a landscape scale, future research should focus on

identify any relationships between attributes at a more local level, trying to classify social groups within caves rather than between them. Such questions could be answered with more frequent records of individuals.

At a landscape scale, future research not only needs to consider where these individual roost are located and the effects they might have on the social network of a population; it also needs to consider the heterogeneity of the landscape (the influences specific habitat types can have on the social structure of a species) and the potential implications this could have if a population lost part of their home range (August *et al.* 2014; He, Maldonado-Chaparro & Farine 2019). Such information could then be combined with habitat suitability models or functional connectivity model (e.g. Chapter 4), to produce outputs which not only take into consideration habitat but also the social relationships between individuals.

7.5. Conclusion and final comments

Urbanisation and agriculture are continuing to grow at a rapid rate, with these alterations to land use having the potential to cause significant negative impacts on biodiversity. To match this, conservation efforts need to continue to utilise and develop new tools, not only examining where species occur but how they move in the landscape. In this thesis, I have combined computer-modelling approaches, a meta-analysis and field experiments to assess how the physical and social structures within an environment can influence the movement and activity of *R. ferrumequinum*, allowing for the creation of better informed practical conservation strategies.

Utilising, developing and incorporating landscape scale approaches into conservation management has been acknowledged to be a progressive way of better protecting species by creating bigger, better and more connected habitats for a wide range of species (Lawton 2010), not just those of conservation concern (Pressey & Bottrill 2009). To achieve such goals and to manage the effects of potential impacts on the environment takes collaborative effort from

many stakeholders, from farmers and environmental groups to universities and national governments. This shift in mind-set is required to take a broader view of the landscape and how individual pressures can cause significant effects outside of their site boundaries. My research has presented novel methods in assessing and predicting the cumulative impacts of anthropogenic pressures on *R. ferrumequinum*, giving a foundation for future research and management plans to achieve the highest biodiversity net gain possible for the species.

Chapter 8 Reference

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I am looking out of my window in an anxious and resentful state of mind, oblivious of my surroundings, brooding perhaps on some damage done to my prestige. Then suddenly I observe a hovering kestrel. In a moment everything is altered. The brooding self with its hurt vanity has disappeared. There is nothing now but kestrel. And when I return to thinking of the other matter it seems less important. And of course this is something that we may also do deliberately: give mention to nature in order to clear our minds of selfish care.

— Iris Murdoch, The Sovereignty of Good